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ION FIXATION BY ALLOPHANE

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Summary

The uptake of cations and anions from salt solutions by allophane has been studied over a range of pH values by means of the equilibrium method previously used for measuring the physical adsorption of cations by this material. Provided account is taken of the pH value of the system prior to analysis, the results obtained are similar to those given by the Schofield net charge method, but minor differences are apparent. Several available procedures have been tested for measuring the cation exchange capacity of soils containing allophane, and the values have been interpreted in terms of the relation between pH and net charge on the colloid as given by the equilibrium experiments.

Considerable fixation of ions by allophane in a water-insoluble form may occur as a result of treatment with suitable concentrated salt solutions. In most cases, the products are amorphous, but acid phosphate and neutral or acid fluoride solutions give a high yield of crystalline product. With alkali metal phosphate solutions the products identified are taranakite minerals, as previously reported by Wada for Japanese allophane. The fluoride solutions give fluoaluminates, the product when sodium fluoride is present in the solution being identical with natural cryolite mineral. The conversion of allophane to fluoaluminates is more rapid than the conversion to taranakites, but it is to be noted that dilute acid fluoride solutions will produce fluoaluminates from kaolin and montmorillonite almost as rapidly as from allophane. Gibbsite, however, as a soil constituent shows considerable resistance to attack by acid fluoride solutions.

INTRODUCTION

It has been pointed out by the writer that allophane will physically adsorb cations from salt solutions, and that the amount adsorbed is dependent on salt concentration and on the effective size of the cation (Birrell and Gradwell, 1956; Birrell, 1961). Recently, Wada and Ataka (1958) have shown that in addition to the physical adsorption process, which they consider has the effect of removing cations and anions from solution in equivalent amounts, ion uptake is also strongly dependent on the pH value

of the system. Working with ammonium chloride solutions of different concentrations, and using a modification of the procedure devised by Schofield (1949) for measuring the net electric charges on clays, they found that at low pH values, the uptake of anion predominated, while as the reaction of the salt solution approached neutrality, the uptake of anion decreased, and that of cation increased, until at pH 7, almost equivalent amounts were taken up.

Wada and Ataka concluded that there were two effects taking place simultaneously, the first being a non-Coulombic salt adsorption which is independent of pH, but which is dependent on concentration, the second being a Coulombic reaction which is largely independent of concentration, but which is strongly pH dependent.

The present writer considered that the equilibrium method originally used to study the salt adsorption effect (Birrell and Gradwell, *op. cit*) would be suitable for studying the second effect also, provided that the pH values of the salt solutions were suitably modified. Although it was found in practice that a certain amount of trial and error was required in order to obtain a reasonably constant pH value over a range of concentrations, the equilibrium method has the advantage of eliminating the large correction due to entrained salt solution inherent in the Schofield method and also avoids possible changes in pH of the system due to the use of a different salt solution as extractant. It appeared of interest to compare the two methods with New Zealand allophane samples.

Base saturation values for soils containing allophane, as calculated from cation exchange capacity values determined by the standard ammonium acetate method in which alcohol is used to remove excess salts, appear abnormally low in relation to the pH values of water suspensions of the soils (Birrell, 1958). One source of error in this method has been shown to be the physical adsorption of ammonium ion by the allophane (Birrell and Gradwell, 1956), a property which is shown also by certain other amorphous materials (Birrell, 1960). Other methods of determining cation exchange capacity have been proposed in which this salt adsorption effect does not occur (Wright, 1934; Mehlich, 1953; Coleman, Weed, and McCracken, 1959). There are, however, considerable differences in the pH values of the salt solutions used to leach the soil in these methods, and it appeared of interest to find if the C.E.C. values so obtained fitted in with the pH dependent type of reaction postulated by Wada and Ataka (*op. cit*).

Further, if their overall picture of the ion uptake mechanism of allophane is correct, the ion preferentially taken up, depending on the pH of the system, should be retained in appreciable amount after washing with water, and may therefore be regarded as "fixed" in some way. Such "fixed" cations should be extractable in part by leaching the water-washed material with dilute acid, and "fixed" anion by a similar extraction with dilute alkali.

Of the anions which can be "fixed" in this way by allophane, phosphate is rapidly rendered insoluble in relatively large amount (Saunders, 1959), and Wada (1959) has shown the rapid conversion of allophane to the

insoluble phosphate mineral "taranakite" by treatment with M ammonium phosphate solution at pH4. It therefore seemed worthwhile to examine "fixation" as a general property of allophane, treating samples of the material with suitable concentrated salt solutions under conditions which would cause either the cation or anion to be fixed, and to examine both the insoluble residue and the solution for reaction products. This comprises the second part of this investigation.

MATERIALS

Fractions containing particles of clay size (less than 2 μ) and clay plus silt size (less than 20 μ) were separated from the Tirau Ash subsoil described previously (Birrell and Gradwell, 1956). The clay fraction was almost entirely amorphous to X-rays, but the clay plus silt fraction showed small amounts of quartz and feldspar in addition to the amorphous material. Dispersion following preliminary hydrogen peroxide treatment was carried out both in alkaline solution and in dilute hydrochloric acid solution at pH 3·7, this latter procedure having been used by Wada and Ataka (*op. cit*).

Dispersion of the soil was obtained by addition of N NaOH to the water suspension until an alkaline reaction to thymolphthalein was obtained. After separating the fractions containing particles of the required size range by centrifuging, flocculation was effected by adding 15 g per litre of sodium sulphate decahydrate where the uptake of chloride, acetate, chromate, or vanadate was to be measured subsequently, or by the addition of 10 g per litre of sodium chloride where sulphate uptake was to be measured. The flocs were then washed once with water, that obtained with sodium sulphate being next washed twice with N/50 sulphuric acid, and that obtained with sodium chloride washed likewise with N/50 hydrochloric acid. Two washings with 90% ethyl alcohol and one washing with acetone followed by drying at a low temperature under an infra-red lamp completed the preparation of the materials.

A fairly good dispersion was also obtained with the Tirau Ash subsoil by adding N HCl to a water suspension until a pH value of 3·7 was reached. The acid concentration was then about N/100. As the acid dispersed clay fraction was to be used for experiments with ammonium chloride, it seemed preferable to disperse with sulphuric acid. This was tried but was found to be ineffective. However, by flocculating the suspension of clay-sized particles with N NaOH (sufficient being added to bring the suspension to the neutral point of bromo-cresol purple) and then washing several times with neutral M/10 sodium sulphate solution, all chloride seemed to have been removed from the material, which was then washed with solvents and dried in the same way as the fractions dispersed in alkaline solution.

A sample (designated SB7562) of a material resembling the "waxy-pan" described by Taylor (1933) was also used as a source of allophane. This material occurred as a lens in sedimentary beds in Raglan County, N.Z. In addition to allophane, the sample contained a small amount of

gibbsite, mostly present as thin sheets around soil aggregates. This material was used after drying at 105°C, without separation of fractions.

For studies on different methods of determining cation exchange capacity and per cent base saturation, the following comparison materials were used in addition to the above:

(1) The Aitutaki soil No. 6168 containing "palagonite", previously described (Birrell and Gradwell, 1956).

(2) Silica-alumina of 18% alumina content, also previously described (Birrell, 1960).

(3) Amorphous alumina prepared by dissolving high purity aluminium strip in caustic soda solution and passing in CO_2 until precipitation was complete. The precipitate was washed twice with water, three times with N/50 HCl, twice with 95% alcohol, and finally with acetone.

Materials containing crystalline clay minerals were also used in experiments with fluoride ion. These were: (1) sample S309, described by Gradwell and Birrell (1954), and consisting of halloysite with a little allophane, (2) a well-characterised halloysite from Matauri Bay, N.Z., supplied by the Director, Pottery and Ceramics Research Association, (3) a kaolin from Wairakei, N.Z., supplied by Dr L. D. Swindale. This sample had been formed by hydrothemal alteration, and consisted of relatively large hexagonal plates. It showed good X-ray and DTA patterns. Finally (4) an acid-washed sample of commercial Wyoming bentonite.

METHODS

The general procedure of Wada and Ataka was used to measure the uptake of ions from solutions of ammonium chloride and potassium chloride by the Schofield method. Five portions of 20 ml of chloride solutions were used per 2 g of sample, and five 20 ml portions of N potassium nitrate for extracting the samples treated with ammonium chloride solution. Samples treated with potassium chloride solution were extracted likewise with N sodium sulphate. The supernatant liquid from the last portion of ammonium or potassium chloride solution applied, was reserved for pH determination. Ammonium was estimated by distillation of liberated ammonia into boric acid solution, potassium by the sodium tetraphenyl boron procedure of Cluley (1955), and chloride by Mohr's method.

In the equilibrium experiments, chloride was determined by Mohr's method, and sulphate gravimetrically. Analyses for other cations and anions followed procedures mentioned previously (Birrell and Gradwell, 1956; Birrell, 1961). In addition, chromate was determined by iodometric titration, and bicarbonate by titration with N/10 HCl using methyl orange as indicator.

For determinations of cation exchange capacity and per cent-base saturation of allopane and comparison materials by alternative methods, those used were as follows:

(1) Leaching with N KCl (pH 6), followed by barium chloride–triethanol–amine (TEA), as described by Coleman, Weed, and McCracken (1959). The barium chloride – TEA reagent was originally proposed by Mehlich (1953) and has a pH of 8·1.

(2) The standard ammonium acetate method as described by Metson (1956), the reagent being adjusted to pH 7·0.

(3) Leaching with N barium acetate solution adjusted to pH 7·0, at the rate of 25 ml per g of material (Wright, 1934).

Electrostatically bonded exchangeable hydrogen and exchangeable Al were determined on the N KCl leachate and exchangeable hydrogen on the $BaCl_2$ – TEA leachate according to the procedure of Coleman *et al.* (*op. cit*).

Exchangeable hydrogen was determined on the N barium acetate leachate, by titrating back to pH 7·0 with N/10 NaOH.

In addition, a C.E.C. value was obtained after treatment (1) above by successive leachings with N/10 $BaCl_2$, water, and 0·6N $CaCl_2$ as prescribed by Mehlich (*op. cit*), the barium in the $CaCl_2$ solution being determined gravimetrically as $BaCrO_4$. The C.E.C. value after treatment (3) was obtained by washing with water until washings were free of Ba^{++}, extracting with the $CaCl_2$ solution and proceeding in the same way.

In order to study the fixation of ions by the soils and soil fractions mentioned in the previous section, 1 g of material was taken per 50 or 100 ml of solution. The suspensions were agitated several times daily at a temperature of 30°C, the solids filtered off into a Gooch crucible, and washed with water until the washings were free of the ion concerned where this was practicable. Complete removal of fluoride ion could not be effected in treatments with ammonium fluoride solution, on account of the appreciable solubility of ammonium fluoaluminate (Seidell, 1940). In treatments with molybdate salts, in some runs washing was stopped short of complete removal of molybdate ion to eliminate any possible solution of iron or aluminium molybdates.

The washed residues were dried in vacuo over calcium chloride and finely pulverised. X-ray analyses were made with Fe $K\alpha$ radiation. Analyses for ions "fixed" by the treatments were carried out by the methods referred to above, or by conventional methods.

RESULTS AND DISCUSSION

pH – Net Charge Relationship of Allophane

MEASUREMENTS OF RELATIVE CATION AND ANION UPTAKES

Table 1 (a) and (b) shows the cation and anion uptakes for two materials of high allophane content by the modified Schofield method using ammonium and potassium chlorides, and Table 1 (c) shows corresponding

values by the equilibrium method using ammonium chloride solutions. Fig. 1 (a) shows results obtained with Tirau clay plus silt fraction for potassium and barium chlorides by the equilibrium method in which concentration was introduced as an extra variable and Fig 1 (b) shows similarly results for potassium and rubidium sulphates. Fig. 1 (c) shows results for potassium acetate, potassium bicarbonate and potassium chromate and bichromate in which pH is the major variant.

TABLE 1—Uptake of Ions by Allophane from Ammonium Chloride and Potassium Chloride Solution

Material	Pretreatment	Strength of Solution	Original pH	Final pH	Uptake, m.e./g	
					Cation	Anion
(a) *Modified Schofield Method* (NH_4Cl)						
Tirau clay fraction ($< 2\ \mu$)	Dispersed with N/100 HCl	N/2	6·95	6·2*	0·31	0·33
		N	3·8	6·35	0·54	0·44
		N	7·7	7·5	0·70	0·39
SB 7562	None	N	7·45	5·9	0·26	0·205
		N	8·0	7·8	0·38	0·20
(b) *Modified Schofield Method* (KCl)						
SB 7562	None	N	5·0	4·9	0·18	0·29
		N	9·0	5·1	0·17	0·25
		N	9·5	6·7	0·26	0·20
		N	10·0	7·2	0·29	0·18
(c) *Equilibrium Method* (NH_4Cl)						
Tirau clay fraction ($< 2\ \mu$)	Dispersed with N/100 HCl.	N/2	4·0	5·3	0·30	0·21
		N	7·4	5·8	0·49	0·36
		N	3·8	6·85	0·57	0·20
SB 7562	None	N	7·4	4·8	0·16	0·17
		N	7·9	5·0	0·14	0·16

*NOTE—Final pH in the results by the modified Schofield method refers to the centrifugate from the last ammonium or potassium chloride treatment (i.e., fifth) except for the value marked with an asterisk which is that of the tenth extract. In the equilibrium method the final pH is that of the system after shaking for one hour.

By plotting the values for net charge (positive or negative) on allophane as deduced from these experiments against final pH as defined earlier, approximate estimates could be made of the pH value at which equivalent amounts of cation and anion are taken up (i.e, net charge = 0). These are set out in Table 2.

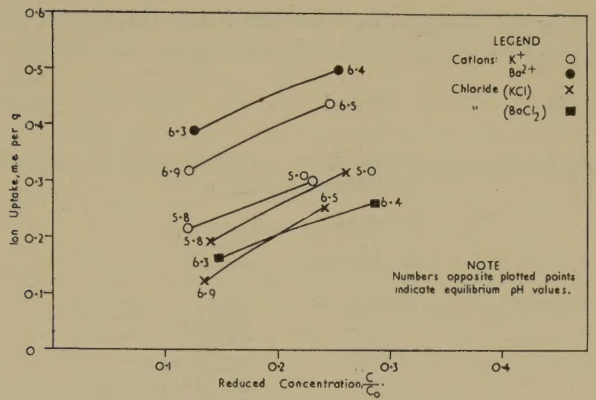

FIG. 1 (a) Uptake of ions from chloride solution by Tirau clay plus silt fraction.

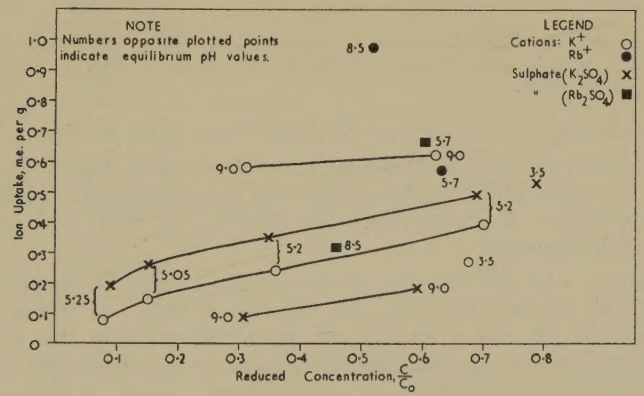

FIG. 1 (b) Uptake of ions from sulphate solution by Tirau clay plus silt fraction.

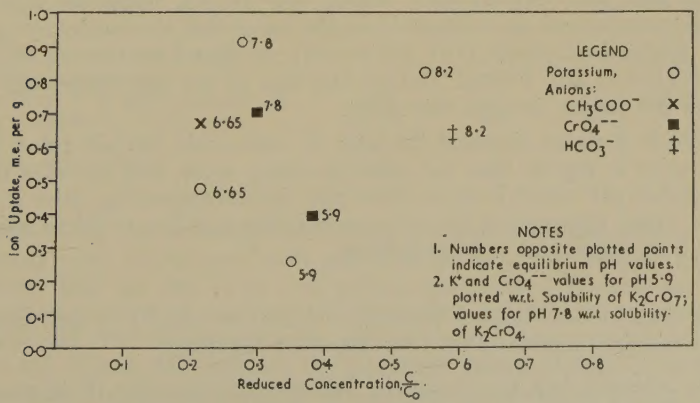

FIG. 1 (c) Uptake of anions from solutions of their potassium salts by Tirau clay plus silt fraction.

TABLE 2—pH Values for Equivalent Ion Uptake (or Zero Net Charge)

Sample	Method	Salt	pH Value for Equivalent Uptake
Tirau Ash, clay fraction	Schofield	NH_4Cl	6·1
	Equilibrium	„	4·9
SB 7562	Schofield	„	5·0
	„	KCl	6·1
	Equilibruim	NH_4Cl	5·0
Tirau Ash, clay plus silt-fraction	Equilibrium	KCl	5·4
	„	K_2SO_4	6·0
	„	Rb_2SO_4	6·0
	„	KOAc and	
	„	$Ba(OAc)_2$*	6·9
	„	K_2CRO_4 and	6·75
		$K_2Cr_2O_7$	

*The results for barium acetate at approximately pH8 (Birrell and Gradwell, 1956) were used in conjunction with the KOAc values in Fig. 1 (c) to establish the required pH value.

The following deductions can be drawn from these results:

1. As shown by the approximately parallel curves in Figs 1 (a) and (b) changes in concentration in the equilibrium experiments have little effect on net charge values. A similar effect was observed by Wada and Ataka using the Schofield method.

2. Reaction with the soil may produce large changes in the pH values of the solutions in both methods (see columns 4 and 5 of Table 1) a fact which should be taken account of when determining the net charge-pH relationship. Strong buffering by the soil in the region pH 5 to pH 7 would appear to be indicated.

3. The pH values for zero net charge (Table 2) given by strong acid-strong base salts such as KCl, K_2SO_4 and Rb_2SO_4 using the equilibrium method correspond approximately to the range for the isoelectric point of New Zealand allophane (pH 5·5 to 6·0), as found by flocculation experiments (Birrell and Fieldes, 1952). The sign of the net charge will reverse as the pH passes through this point.

4. Table 2 shows also that for salts of weak acids, the pH value for zero net charge is higher than for salts of strong acids, and for salts of weak bases, this pH value is lower than for the corresponding salts of strong bases. These changes are in the expected direction for a colloid which can show both acidic and basic properties.

MEASUREMENT OF CATION EXCHANGE CAPACITY (C.E.C.) AND PER CENT BASE SATURATION (B.S.) BY VARIOUS METHODS

The relevant data are set out in Tables 3 (a) and (b). It is assumed in calculating the per cent B.S. figures in Table 3 (b) that the same values for exchangeable Ca and Mg are given by N KCl and N Ba $(OAc)_2$ as by

2N NH_4OAc. The good agreement for exchangeable Ca and Mg in the case of the Aitutaki soil using either 2N NH_4OAc. or N KCl supports this assumption.

TABLE 3 (a)—Base Exchange Data for Tirau Ash Subsoil and Related Materials by Various Methods

(values in m.e. per 100 g)

Material	Leaching Solution	Total Bases	Exchangeable Bases Ca	Mg	H	Al	C.E.C. (Direct Values)
Tirau Ash subsoil	2N NH_4OAc	6·7	2·7	1·8			(See 3b)
	N KCl				0·3	2·0	
	$BaCl_2$–TEA				22·0		22·6
	N $Ba(OAc)_2$				15·0		16·3
Silica-alumina (18% Al_2O_3)	N KCl		Nil	Nil	8·0	34·4	
	$BaCl_2$–TEA				41·4		73·0
Amorphous Al_2O_3	N KCl		Nil	Nil	4·4	Nil	
	$BaCl_2$–TEA				39·3		20·3
Aitutaki Soil no. 6168	2N NH_4OAc	82·1	39·0	45·4			93
	N KCl		38·4	42·5	Nil	Nil	
	$BaCl_2$–TEA				13·3		90

TABLE 3 (b)—C.E.C. and % B.S. Values for Tirau Ash Subsoil by Various Methods

Method	C.E.C.	% B.S.	Calculation of % B.S. as
2N NH_4OAc (80% alcohol wash)	34·0	20	T.B./C.E.C.
2N NH_4OAc (water wash)	8·0	56	,, ,,
Ca + Mg + Al + H (KCl extract)	6·8	66	Ca + Mg/C.E.C.
Ca + Mg + H($BaCl_2$–TEA)	26·5	17	,, ,,
Ca + Mg + H(N BaOAc)	19·5	23	,, ,,
$BaCl_2$–TEA, direct value	22·6	20	,, ,,

Comparison of the exchangeable hydrogen values obtained with N KCl (pH approx. 6·0) on the one hand and with $BaCl_2$ – TEA reagent on the other shows that for the Tirau Ash subsoil, the silica – alumina and the amorphous alumina, there is a large pH dependent charge mobilised between pH 6 and pH 8·1. Similarly results obtained using barium acetate show that for the Tirau Ash subsoil, the negative charge mobilised between pH 6 and pH 7 is also quite significant. The additional charge arising when the pH increases from 7·0 to 8·1 is, however, less than would be expected from previous results using barium acetate alone (Birrell and Gradwell,

1956), but it is to be noted that in the present experiments, the buffering action of triethanolamine has been introduced as an additional factor. Nevertheless, for this subsoil, the amount of negative charge mobilised between pH 6 and pH 8 is greater than for the soils examined by Coleman, Weed, and McCracken (*op. cit*).

The direct C.E.C. values in col. 8 of Table 3 (a) are in agreement with the findings of Wada and Ataka (*op. cit*) and with the results discussed in the previous section in so far as they show that increasing pH causes allophane to retain increasing amounts of cation in a non-water-soluble form. Similar properties are shown also by co-precipitated silica – alumina and by amorphous alumina. It is also apparent that if the sum of bases in the N KCl extract (including H and Al) is taken as the true C.E.C. for allophane, values for C.E.C. much in excess of this may be obtained if the soil is leached with salt solutions at pH values of 7 or more, even when the salt adsorption effect is eliminated.

Considering the per cent B.S. figures in Table 3 (b), the value of 66% obtained from the experiments with N KCl appears to be the most reasonable in view of the fact that the pH of the Tirau Ash subsoil in water suspension is 5·9. However, the value obtained by using 2N NH_4OAc with water-washing is only slightly less, but two partly compensating errors are probable in this latter value. In the first place, NH_4OAc should give a higher value for C.E.C. than N KCl due to the higher pH as discussed above. In the second place there will be hydrolysis of the ammonium exchange complex by water, leading to a reduction in C.E.C. The magnitude of this second effect cannot be readily estimated, because the alternative procedure of washing with alcohol introduces a large error due to salt adsorption, as already mentioned. Evidence of the hydrolysis effect may be obtained by noting that when N $Ba(OAc)_2$ is used, the C.E.C. as measured by the retained barium is less than the sum of bases plus hydrogen (Table 3 (a)).

If it is desired to measure the true C.E.C. of allophane soils, the use of N KCl solutions in the manner suggested by Coleman *et al.* would seem to offer the best practical approach.

It is clear from Table 3 (a) that "palagonite" although amorphous, resembles montmorillonite in its base exchange properties rather than allophane.

Nature of Products Formed From Allophane by Ion Fixation

Evidence for Fixation of Ions

The suspensions of Tirau clay plus silt fraction (6g) in rubidium sulphate solutions at pH values of 5·7 and 8·5 (see Fig. 1b) after removal of aliquots for analysis were filtered and the soil fraction washed with 300 ml water. The material was then leached with 100 ml N acetic acid. The same treatment was also given to two suspensions of the same soil fraction in M potassium bicarbonate solution. The amounts of cation in the acetic acid extract as percentages of the total cation taken up are as follows:

Salt Solution	Equilibrium pH	% Cation in Acetic Extract
M Rb_2SO_4	5·7	8·3
1·25 M Rb_2SO_4	9·5	30
M $KHCO_3$	8·6	35
,,	8·2	29

It may be deduced from these results that alkaline conditions favour the "fixation" of cation in a form insoluble in water, and that some, at least of the fixed cation is soluble in dilute acid. It appears that studies of the fixation of cations by allophane should be carried out at pH values of 8 or more.

There is strong evidence to suggest that, in general, the "fixation" of anions by allophane will be favoured by slightly acid conditions. Wada and Ataka (*op. cit*) found the retention of chloride at pH 5 to be several times the retention of ammonium at the same pH. In the present investigation the uptake of sulphate from potassium sulphate solution at pH 3·5 was about double the uptake of potassium, and of the sulphate retained by the allophane in a form insoluble in water, 53% was soluble in N/2 NaOH. Saunders (1959) studied the retention of phosphate by a soil derived from andesitic volcanic ash, and containing allophane as the dominant colloidal constituent. He found the retention to be greatest between pH 3·5 and 7·0, falling off rapidly above and below this range. Wada (1959) found the formation of taranakite from allophane to proceed much faster at pH 4 than at pH 7.

Hence it seemed that "fixation" of anions by allophane should be studied in systems where the pH value was about 4, or as close to this as the stability of the salt solutions would permit.

EXAMINATION OF ALLOPHANE SAMPLES CONTAINING "FIXED" IONS

Potassium

The Tirau Ash clay plus silt fraction after contact for three weeks at 30°C with M potassium acetate solution at pH 9·0 was washed until free of water-soluble potassium, and when vacuum-dried showed a gain in weight of 26%. The ammonium acetate method (Metson, 1956) showed that the treated soil fraction contained 60 m.e. per 100 g exchangeable potassium. X-ray analysis of the product gave lines corresponding to spacings of 1·432, 2·03, and 2·34 Å which could not be identified with any likely potassium compound, but which were stronger than the identifiable lines in the product due to quartz and feldspar. The above-mentioned spacings are similar to those given for synthetic lithium and magnesium spinels in the X-ray Powder Index File (1958), but no data are listed for a corresponding potassium spinel structure.

Treatment with 4M potassium acetate solutions at pH 9 under the same

conditions gave a product which required much more washing to free it of soluble potassium, and which was amorphous to X-rays. It contained 6·40% of K_2O (136 m.e. per 100 g) of which 24 m.e. per 100 g was extractable with N ammonium acetate solution. The material, however, was not a true exchanger, as the C.E.C. value by the ammonium acetate method with alcohol washing was 49 m.e. per 100 g but only 7 m.e./100 g with water washing. The weight of the sample was almost unchanged by the treatment.

A more alkaline solution of M potassium acetate solution (pH 10) also gave an amorphous product. In this case there was an overall loss of 12%, considerable loss of alumina, and it was not possible to wash the product free of water-soluble potassium.

It may be concluded that moderately alkaline solutions cause allophane to fix cations, partly in a readily exchangeable form, but under more strongly alkaline conditions the material is attacked.

Phosphate

Visual changes in the Tirau Ash clay fraction when treated with either ammonium or potassium dihydrogen phosphate (molar solutions) at about pH 4·0 were similar to those observed by Wada (*op. cit*) who obtained the phosphate mineral "taranakite" by treating Japanese clays containing allophane or halloysite with ammonium phosphate solution under similar conditions. The products obtained in the present investigation could be readily washed free of soluble phosphate with water. After drying in vacuo, X-ray analyses of the products obtained with both phosphate solutions agreed well with the values given by Murray and Dietrich (1956) for a specimen of taranakite containing potassium as the dominant base, as shown below.

Tirau Clay Treated With				Taranakite from Pig Hole Cave (Murray and Dietrich)	
M $NH_4H_2PO_4$		M KH_2PO_4			
d	rel. I	d	rel. I	d	rel. I
15·60	0·80	15·60	1·00	15·49	1·00
7·93	0·88	7·82	0·20	7·82	0·23
7·45	0·84	7·40	0·32	7·43	0·18
5·91	0·56	5·88	0·40	5·82	0·13
4·26	0·85	4·31	0·40	4·27	0·12
4·02	0·56	4·02	0·10	4·00	0·03
3·82	1·00	3·81	0·90	3·79	0·25
3·76	0·64			3·72	0·11
3·36	0·68	3·36	0·25	3·34	0·10
3·15	1·00	3·13	0·80	3·12	0·19
2·83	0·68	2·82	0·50	2·82	0·12
2·63	0·48	2·62	0·40	2·62	0·12

The weight changes and partial analyses of the products after reaction for 3 weeks at 30°C were as follows:

	M $NH_4H_2PO_4$	M KH_2PO_4
Gain	95%	89%
P_2O_5	31·0%	30·1%
$(NH_4)_2O$	4·42%	.....
K_2O	.....	9·10%
SiO_2	12·33%	14·46%

Molecular ratios $(NH_4)_2O$: $P_2O_5 = 0{\cdot}39$; K_2O: $P_2O_5 = 0{\cdot}455$.

These ratios are approximately the same as the value found by Murray and Dietrich (*op. cit*) for a specimen of taranakite formed from a brecciated clay.

If the results above are recalculated so as to represent a silica-free product the P_2O_5 contents become 35·3 and 35·2% respectively. These values are only a little lower than those for the two taranakite samples analysed by Murray and Dietrich.

On petrological examination, the material was seen to consist partly of irregular grains with no birefringence and refractive index of 1·516 (± ·002). There were also present isolated prismatic crystals for which refractive indices ranged from 1·510 to 1·518 (all ± ·002). These values are in reasonable agreement with those given by Murray and Dietrich for the natural mineral, and by Wada for his phosphate treated allophane.

Comparison of the analysis of the phosphated allophane with that of the original untreated sample shows that about 25% of the silica has gone into solution after treatment with ammonium phosphate, and about 15% after treatment with potassium phosphate. However, in view of the ease with which allophane is attacked by acid solutions (Birrell and Fieldes, 1952) the main reaction is likely to be solution of the alumina, followed by precipitation of the taranakite, as suggested by Kittrick and Jackson for kaolin (1956). The Murray and Dietrich formula for taranakite, on the assumption that the silica is largely unattacked, would require that free alkali be produced as a result of the reaction of the phosphate solution with allophane. This would explain the retardation of the reaction at pH 7·0, as observed by Wada (*op. cit*).

Molybdate

The product obtained by digesting the clay plus silt fraction of the Tirau Ash subsoil with an M/4 solution of ammonium paramolybdate $(NM_4)_6 Mo_7O_{24}$, $4H_2O$, saturated with MoO_3, and having a pH of 5·6, appeared to be much contaminated with a white crystalline solid. However, by prolonged washing with water, continued until only a trace of molybdate ion

could be detected in the washings with lead acetate solution, a product was obtained which contained 5·6% of MoO_3, but which on X-ray analysis proved to be amorphous.

According to Thorne and Roberts (1948), concentrated alkali paramolybdate solutions are unstable and precipitate sparingly soluble trimolybdates. Analysis of the crystalline material deposited in large amount from the ammonium paramolybdate solution in a blank run showed that its composition corresponded to that of ammonium trimolybdate monohydrate.

There is the possibility that crystalline iron or aluminium molybdates may also be formed by treatment of allophane with ammonium molybdate solutions, but that they may be soluble enough to be washed out before the product can be freed of soluble molybdate ion. However, the synthetic iron and aluminium molybdates which have been examined by Jones (1957) appear to be sparingly soluble. Jones found by X-ray evidence that the latter substance was also formed by treating boehmite or halloysite with a sodium molybdate solution of pH 4·5 to 5·5.

X-ray data for ammonium trimolybdate or other ammonium polymolybdates were not found in the literature, but two preparations which differed in the amount of substance separating from the ammonium paramolybdate solution gave the X-ray spacings shown in Table 4, cols. 2 and 3. In column 1 of this table are shown also the spacings for a partly-washed allophane treated with this solution. It is very improbable that there is any new crystalline phase present in the treated allophane, and if these results are compared with those given in the reference cited above for synthetic iron and aluminium molybdates, there is little likelihood of identifying such materials in a product which is contaminated by precipitates arising from the alteration of ammonium paramolybdate.

Solutions of sodium molybdate made slightly acid by addition of solid molybdic acid are also unstable, but the precipitates formed in them gave identical X-ray patterns over a fairly wide range of pH and concentration. The X-ray pattern of these precipitates is given in column 4 of Table 4. The solubility of this material lay between 0·2 and 1·0 g per 100 ml, and its composition corresponded approximately to $Na_2Mo_6O_{19}$, $4\frac{1}{2}$ H_2O. The spacings obtained would not appear to preclude the identification of the iron or aluminium molybdates described by Jones, even if the product were much contaminated by such precipitates.

Three treatments of allophane using sodium molybdate solutions were carried out at 30°C. Particulars of these are tabulated below:

Concen. With Respect to MoO_3	pH	Soln. Volume in ml per g of Clay Fraction	Time of Treatment	MoO_3 Content of Product %	Loss in Weight %
0·15M	5·1	100	14 days	8·3	33
0·70M	6·2	26	4 days	3·03	2
1·10M	6·4	50	14 days	7·5	25

TABLE 4—X-ray Powder Data for Allophane Treated with Ammonium Paramolybdate Solution, and for Precipitates Formed in Acid Solutions of Ammonium Paramolybdate and Sodium Molybdate

Allophane Treated With Paramolybdate Partly Washed		Precipitate From Paramolybdate Soln (Large Amt.)		Precipitate From Paramolybdate Soln (Small Amt.)		Precipitate From Acid Sodium Molybdate Solns	
d	rel. I	d	rel. I	d	rel. I	d	rel. I
10·5	0·80			9·56	0·90		
8·8	1·00	8·94	1·00				
7·03	1·00	7·87	0·90			7·60	1·00
		7·12	0·30	7·12	0·05		
				6·46	0·10	6·73	0·10
5·91	0·15			5·91	0·30	5·85	0·15
5·79	0·40	5·79	0·10				
5·25	0·45	5·45	0·05			5·43	0·05
4·89	0·15	5·00	0·10			5·01	0·15
4·39	0·10	4·43	0·15	4·04	0·15	4·40	0·05
3·69	0·15	3·74	0·35			3·78	0·10
3·63	0·15	3·65	0·05				
3·55	0·40	3·57	0·20			3·57	0·05
3·46	0·05	3·47	0·20				
3·27	0·40	3·42	0·10	3·30	0·25	3·38	0·30
3·13	0·05			3·17	1·00	3·12	0·60
3·08	0·10	3·03	0·10			3·01	0·20
2·89	0·25	2·95	0·40	2·92	0·10	2·87	0·20
2·73	0·05	2·78	0·20			2·81	0·05
2·64	0·10						
2·57	0·10					2·59	0·10
2·53	0·05	2·47	0·10				
2·41	0·20					2·44	0·10
2·30	0·10	2·22	0·10			2·32	0·10
2·08	0·10			2·13	0·05	2·25	0·10
2·06	0·10			2·018	0·10	1·90	0·15
1·986	0·20	1·84	0·10			1·655	0·05
1·76	0·20	1·76	0·05	1·684	0·10	1·62	0·15
		1·555	0·15			1·525	0·10

It was noted that the amount of white precipitate formed in the clay suspension as a result of decomposition of the sodium molybdate solutions was much less than in the blank molybdate solutions run simultaneously. The clay after treatment was given a moderate amount of washing, and in all cases the product was amorphous to X-rays, there being also no detectable contamination due to decomposition of the sodium molybdate. It is concluded therefore that treatment of allophane with alkali molybdate solutions of pH value greater than 5·1 does not lead to a crystalline product although there is marked fixation of molybdate.

Chromate, Vanadate, and Sulphate

Fixation of chromate anion was studied by treatment with 0·8M ammonium dichromate solution (pH 4·5), made by dissolving the required amount of A.R. grade salt in water. To study the action of vanadate anion, a M solution of sodium metavanadate was prepared from the commercial salt

and shaken for one hour with excess vanadium pentoxide which was then filtered off, giving a solution of pH 6·5. The corresponding sulphate solution was 0·47M potassium sulphate brought to pH 3·9 by addition of sulphuric acid. All three treatments when applied to the Tirau Ash clay plus silt fraction for a period of three weeks at 30°C, gave products which on X-ray analysis showed only the lines of quartz and feldspar present in the original soil fraction. The products formed must therefore be regarded as amorphous. Partial analyses of the chromate and vanadated treated samples are given below.

	Original-Soil Fraction	Chromate Treated	Vanadate Treated
Loss in weight %	—	35	13
SiO_2	58·6	54·2	58·5
Al_2O_3	30·7	33·2	—
Fe_2O_3	6·28	6·6	—
Cr_2O_3	—	1·74	—
V_2O_5	—	—	3·25
SiO_2/Al_2O_3	3·24	2·77	—

The chromate treated sample shows a lower silica content than the original soil fraction, but there is also a general loss of other constituents. With vanadate treatment, the general loss is smaller, and the silica content is unaltered. The potassium sulphate treatment resulted in a loss of 53% by weight of the sample.

Fluoride

It was shown by Turner and Rice (1952) that neutral ammonium fluoride solution will displace phosphate adsorbed by aged aluminium hydroxide gel, with the simultaneous formation of ammonium fluoaluminate $(NH_4)_3AlF_6$, which is sparingly soluble and highly stable in alkaline solution. Pender (1959) worked out conditions necessary for the quantitative precipitation of aluminium from solution in dilute hydrofluoric acid by sodium fluoride. The precipitate formed is a sodium fluoaluminate of the composition 11 NaF, $4AlF_3$. It seemed likely, therefore, that neutral or acid fluoride solutions, on account of the high stability of fluoaluminates, would react with taranakite in spite of the low solubility of this mineral in water, stated by Hasemann, Brown, and Whit (1950) as being two to four parts per million.

When ammonium taranakite, prepared from the Tirau allophane, as described earlier, was shaken occasionally over a period of three hours at room temperature with 3M NH_4F solution, contained in a polythene bottle, the mineral lost much of its opacity, and about 75% of the phosphate in the taranakite went into solution. The solid residue, after washing with a small quantity of water, and drying in vacuo, gave an X-ray pattern closely resembling that of the synthetic ammonium fluoaluminate prepared by Turner and Rice (*op. cit*). Another specimen of taranakite, treated under

the same conditions with a solution which was M/2 with respect to both NaF and HF, became almost transparent and gave an X-ray pattern which showed the dominant lines of natural cryolite, Na_3AlF_6. The detailed results of X-ray analysis of the products arising from both these treatments are shown in Table 5. The prominent lines due to spacings of 15·6, 7·9, 7·45, and 5·9 Å in the original taranakite were absent from the X-ray patterns given by both products. According to solubility figures given by Seidell (1940), both ammonium fluoaluminate and cryolite are more soluble in water than taranakite.

TABLE 5—X-ray Analyses of Taranakite Treated with Fluoride Solutions, and of Tirau Clay Fraction Treated with Neutral Fluorides

Taranakite + M/2 $NaHF_2$		Tirau Clay + M NaF		Cryolite (natural)*		Tirau Clay + 5M NH_4F		$(NH_4)_3AlF_6$ (Turner & Rice)	
d	rel. I	d	rel. I	d	rel. I	d	rel. I	d	rel. I
				5·10	0·05	5·16	1·00	5·16	1·00
4·50	0·55	4·53	0·50	4·52	0·30	4·46	0·60	4·47	0·63
4·43	0·55	4·43	0·50	4·43	0·30	3·16	0·60	3·16	0·57
3·885	0·60	3·88	0·45	3·885	0·65	2·59	0·30	2·59	0·27
3·46	0·10			3·46	0·10	2·57	0·30		
				2·79	0·40	2·24	0·50	2·24	0·42
2·74	0·85	2·75	1·00	2·74	0·85	1·72	0·20		
2·43	0·15	2·43	0·20	2·45	0·30		Taranakite		
2·33	0·55	2·33	0·50	2·32	0·40		+ 3M NH_4F		
2·26	0·20			2·265	0·20		d	rel. I	
				2·22	0·10		5·11	0·50	
2·15	0·20	2·18	0·25	2·15	0·15		4·41	1·00	
2·09	0·20			2·09	0·15		3·11	1·00	
1·941	1·00	1·94	1·00	1·941	1·00		2·65	0·05	
				1·79	0·05		2·535	0·20	
				1·76	0·05		2·20	0·75	
1·74	0·15			1·737	0·10		1·97	0·20	
1·719	0·10			1·719	0·20		1·79	0·20	
1·68	0·05			1·674	0·08		1·69	0·15	
1·601	0·15			1·594	0·15		1·55	0·20	
1·573	0·30	1·573	0·35	1·566	0·40				

Sample from Greenland, supplied by Ward's Natural Science Museum, New York, U.S.A.

In view of the above facts, it seemed probable that allophane itself might be converted to alkali fluoaluminates by treatment with neutral fluoride solutions. To test this, a sample of Tirau Ash clay fraction was shaken occasionally over a period of ten days at room temperature with a 5M solution of neutral ammonium fluoride. After the first day, the suspension was aspirated to remove ammonia, but no further aspiration was carried out. The product could not be washed free of fluoride as tested with calcium chloride solution, but when it was judged that the highly soluble ammonium fluoride had been removed, it was dried in vacuo.

Another sample of the same clay fraction was treated similarly with M sodium fluoride solution (omitting the aspiration) and gave a strongly alkaline suspension, but in this case the product could be washed free of fluoride ion.

The vacuum-dried materials were finely ground and on X-ray analysis gave the results shown in Table 5, from which it can be seen that allophane gives the same fluoaluminate as taranakite in the presence of the appropriate alkali fluoride.

The reaction of allophane with neutral fluoride solutions is more rapid than with phosphate solutions, as Wada (*op. cit*) failed to obtain clear X-ray patterns by reacting allophane with ammonium phosphate solution at pH 7 for three weeks, and the pH in the reaction with neutral fluoride solution obviously rises much above this value. Simple stoichiometric considerations indicate that relatively more alkali will be produced in the formation of fluoaluminates from allophane than in the formation of taranakite. Consequently, in an acid medium, the reaction with fluoride should be greatly accelerated, which proved to be the case. When a 1 g sample was treated with 100 ml of solution corresponding to M/2 $NaHF_2$, the appearance of the allophane changed markedly within 48 hours, becoming lighter in colour, much less opaque, and, in fact, difficult to see in the suspension owing to the low refractive index of the cryolite. Analysis of the product from the Tirau Ash clay fraction after treatment for five days at 30°C with the above-mentioned fluoride solution gave the following results:

F;	43·0%
Na;	26·1%
Al;	10·0%
Fe;	0·84%
H_2O below 105°C;	5·75%
H_2O above 105°C;	9·7%
Total:	95·49%

For the anhydrous substance, this analysis indicates the formula $Na_{3\cdot05}AlF_{6\cdot1}$, corresponding closely to that of natural cryolite. This is well supported by the X-ray evidence, as can be seen by comparing the results for the allophane samples in Table 6 with the spacings of natural cryolite given in Table 5.

In addition, the above product resembled natural cryolite in being easily fusible in the Bunsen flame. The refractive index value was 1·34, in close agreement with the mean value of 1·338 for the mineral, as stated by Palache, Berman, and Frondel (1951). On the basis of fluorine content, the water-free substance prepared from allophane would contain 94 per cent cryolite.

X-ray data in Table 6 indicate that several common crystalline clay minerals also show alteration to cryolite in a short space of time when treated with the NaF + HF solution mentioned above. Gibbsite is, however, rather resistant. This was confirmed by carefully hand-picking pieces of gibbsite from the original soil (SB7562) and treating them with the fluoride reagent. In the product, X-ray analysis showed all the prominent lines of gibbsite, with only the 2·74 Å reflection of cryolite visible, but this had very probably come from allophane contaminating the gibbsite fragments.

TABLE 6—X-ray Analyses of Products Formed by Treating Samples Containing Various Clay Minerals with M/2NaF + M/2HF Solution

Allophane 2 Days		Allophane 5 Days		Allophane Plus Gibbsite 6 Days		Halloysite 6 Days		Kaolin 6 Days		Bentonite 3 Days	
d	rel. I	d	rel. I	d	rel. I	d	rel. I	d	rel. I	d	rel. I
						8·67	0·18	7·82	0·05		
		5·00	0·10	4·80+	0·30			4·97	0·10		
		4·52	0·15					4·53	0·60	4·53	0·40
4·46	0·50	4·43	0·50	4·43	0·60						
4·20*	0·10	4·23*	0·30	4·17*	0·20						
3·86	0·63	3·88	0·50	3·86	0·70	3·86	0·75	3·88	0·72	3·88	0·70
		3·48	0·10	3·46	0·05			3·48	0·10	3·48	0·10
3·30*	0·10	3·32*	0·15	3·30*	0·20	3·32*	0·05				
		3·04**	0·18	3·03**	0·10			2·98**	0·20	2·97**	0·05
2·73	0·80	2·75	0·86	2·73	1·00	2·73	0·97	2·76	0·80	2·74	0·90
2·43	0·10	2·43	0·20	2·43	0·20	2·43	0·18	2·44	0·20	2·43	0·20
2·32	0·50	2·34	0·28	2·32	0·50	2·33	0·52	2·34	0·55	2·33	0·60
2·28	0·50	2·27	0·16	2·27	0·10	2·26	0·18	2·27	0·20	2·27	0·20
		2·22	0·08	2·21	0·05	2·22	0·05	2·24	0·10	2·22	0·05
2·14	0·13	2·16	0·10	2·14	0·15	2·14	0·15	2·16	0·20	2·15	0·20
2·085	0·13	2·10	0·10	2·08	0·15	2·085	0·10	2·09	0·20	2·09	0·20
1·94	1·00	1·941	1·00	1·94	1·00	1·94	1·00	1·94	1·00	1·94	1·00
1·793	0·20	1·798	0·10	1·79	0·15	1·80	0·05	1·793	0·05	1·76	0·05
1·732	0·20	1·74	0·10	1·732	0·10	1·732	0·15	1·741	0·15	1·736	0·10
1·594	0·20	1·596	0·15	1·590	0·25	1·594	0·15	1·598	0·10	1·598	0·15
1·569	0·40	1·573	0·50	1·569	0·45	1·569	0·25	1·576	0·35	1·569	0·40

+Gibbsite *Quartz **Feldspar

Montmorillonite is, however, much more resistant to alteration by neutral ammonium fluoride solution than is allophane. A sample of Wyoming bentonite treated with a M solution of this reagent for 3 weeks at 30°C gave a product showing only a few minor lines in the X-ray pattern which could be attributed to ammonium fluoaluminate. The original basal spacing of montmorillonite was, however, very strong in the X-ray of the treated material.

The greater ease with which allophane is attacked by this reagent can be attributed to its very high external surface (Birrell and Gradwell, 1956) and the labile nature of the aluminium (Birrell and Fieldes, 1952).

The comparatively rapid rate of reaction of allophane and halloysite with acid fluoride solution is shown by the following analytical figures (on a water-free basis):

Material	Minerals	Time	Wt. Gain	F%	Na%	Al%
Tirau Ash $< 2\ \mu$	Allophane	2 days	72%	50·9		
Tirau Ash $< 2\ \mu$	Allophane	5 „	78 „	50·9	31·4	12·6
SB 7562	Allophane, gibbsite	8 „	90 „	48·2		12·8
S 309	Halloysite, allophane	12 „	70 „	50·2		

The lower percentage of fluorine relative to aluminium shown by sample SB7562 is almost certainly due to unattacked gibbsite, as mentioned earlier. For the other samples tabulated above, the fluorine content indicates 90% conversion to cryolite.

From the analysis of the original allophane sample, and that of the product obtained from it after treatment for five days with M/2 $NaHF_2$ solution, it can be shown that at least 75% of the silica originally present in the allophane has gone into solution. This represents a much greater dissolution of silica than in the treatment of allophane with an acid ammonium phosphate solution described earlier. Qualitatively, the presence of silica can be shown in the precipitates obtained when excess ammonium carbonate solution is added to the fluoride solutions after treatment of the soil fractions or clay minerals mentioned in this investigation.

CONCLUSIONS

For studying the pH-net charge relationship of highly buffered soil constituents such as allophane the equilibrium method offers certain advantages as a research procedure over the modified Schofield method. Using the equilibrium method, the isoelectric point of the colloid will be given by the pH value of the system when equivalent amounts of cation and anion are taken up from strong acid-strong base salts. In order to avoid

errors due to salt adsorption effects, and to the mobilisation of negative charges by allophane at pH values above its isoelectric point, the cation exchange capacity of soils containing allophane should be obtained by summation of exchangeable bases, hydrogen, and alumina in a leachate of the soil using an unbuffered solution such as N KCl, originally at about pH 6. The quantities mentioned above may be conveniently determined according to the procedure of Coleman, Weed, and McCracken (*op. cit*). The retention of cations in a non water-soluble form by allophane soils increases markedly as the pH of the salt solution increases from the natural soil pH up to about pH 9, an effect which may have some application for increasing the retention of potassium by volcanic ash soils. The conversion of allophane to crystalline aluminium minerals in high yield by treatment with salt solutions would appear to require either that the product be very insoluble, as with the taranakites, or be sparingly soluble as well as stable over a fairly wide range of pH values, as with the fluoaluminates.

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THE GEOCHEMISTRY OF BROMINE AND IODINE IN NEW ZEALAND THERMAL WATERS

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Summary

A survey has been made of the chloride, bromide, and iodide concentrations in waters from many springs and drillholes in hydrothermal areas of New Zealand.

The Cl/Br atomic ratios for neutral sodium chloride waters are of similar order (650 to 850) throughout the rhyolite pumice and ignimbrite region of the North Island, but in active andesite volcanic areas, acid waters heated by low-pressure, high-temperature volcanic steam have high Cl/Br ratios. Bromide and iodide concentrations are relatively high in waters from areas of sedimentary rocks.

Iodide concentrations are commonly in the range 0·3 p.p.m. to 5 p.p.m in the volcanic areas, and have little relationship to chloride contents.

For waters from the many drillholes at Wairakei the ratio Cl/Br is constant over the field, and has not changed in five years.

Introduction

Previous studies in this Laboratory (Golding and Speer 1961; Ritchie, 1961) have shown that definite relationships exist between trace constituent concentrations and ratios in New Zealand thermal waters and their geological environment. The variations in chloride, bromide, and iodide concentrations in thermal waters are now examined.

Some facts concerning chlorine, bromine, and iodine geochemistry may be summarised. These three halogen ions have a range of ionic radii (Cl = 1·81 Å, Br = 1·96 Å, I = 2·20 Å) and of polarisabilities which would suggest a gradation in their abilities to substitute for ions such as OH^- in silicate minerals. Goldschmidt (1954) stressed of the ability of iodide to substitute for hydroxyl ions. From the values summarised by Correns (1956), average concentrations of chlorine, bromine, and iodine in igneous rocks are about 220 p.p.m., 3·3 p.p.m., and 0·3 p.p.m. Average atomic ratios Cl/Br and Cl/I are therefore about 150 and 2,500.

Bromine and iodine are concentrated with respect to chlorine in plant and animal material, particularly marine organisms. Sedimentary rocks, as a consequence, usually have Cl/Br and Cl/I ratios rather lower than the average for igneous rocks. For example, the average Cl/Br ratio for 17 German greywackes was reported as 19 by von Engelhardt (1936), while Behne (1953) suggested an average Cl/Br ratio of 90 for sediments. Oil-field brines commonly contain high bromide concentrations, as do many natural spring waters which have percolated through, and partially leached, organic strata in sedimentary rocks. Soils also tend to concentrate bromine and iodine.

The relative ease of oxidation of iodide, and to a lesser extent bromide, allows their separation from chloride in many natural processes. The standard electrode potentials (Latimer, 1952) for reactions of type, $2X^- = X_2 + 2e$ is, for X = Cl, − 1·3595 volts; for X = Br, — 1·0652 volts; for X = I, − 0·5355 volts. The reference potentials for two further reactions are of interest.

$$2H_2O = O_2 + 4H^+ + 4e \qquad E° = -1{\cdot}229 \text{ volts}$$
$$2H_2O = O_2 + 4H^+ (10^{-7}\text{M}) + 4e \qquad E = -0{\cdot}815 \text{ volts}$$

$$\tfrac{1}{2} I_2 + 3H_2O = IO_3^- + 6H^+ + 5e \qquad E° = -1{\cdot}195 \text{ volts}$$
$$\tfrac{1}{2} Br_2 + 3H_2O = BrO_3^- + 6H^+ + 5e \qquad E° = -1{\cdot}52 \text{ volts}$$

This free energy information in the form of electrode potentials shows that in neutral solutions iodide can be oxidised by air to iodine, but not bromide or chloride to their elements. In acid solution it is possible for bromide to be oxidised to bromine by aerial oxidation. Iodate could be formed from iodide, particularly in neutral to alkaline solution. For example, the Chilean nitrate deposits contain iodine in the form of iodate.

The ratios Cl/Br and Cl/I for marine air masses are very much lower than for sea-water. Inland rain water may have higher Cl/Br and Cl/I ratios than the first rain deposited from an air mass in the coastal areas (Rankama and Sahama, 1949). Oxidation of iodide may account for this element's volatility, but the form in which bromine is volatilised is not known.

Chlorine, bromine, and iodine are at present being liberated into the earth's atmosphere by volcanic activity. For example, 1 p.p.m. iodide was found in ammonium chloride sublimates at Vesuvius (Stoklasa, 1927). In a review article on volcanic exhalations, Naboko (1959) summarised more recent Russian work on the bromine and iodine contents of volcanic vapours and sublimates.

It is possible that the concentrations of the halogens on the earth's surface may, in part, have resulted by the formation of the sea from the condensation of a primordial atmosphere. This theory provides an alternative, or an amendment, to the theory that the oceans have grown throughout the history of the earth from contributions of water and halogens from volcanic sources. This point will be discussed at the end of the paper.

As the sea contains most of the bromine (concentration, 65 p.p.m.) and chlorine (18,980 p.p.m.) on the earth's surface, and about half of the iodine (0·05 p.p.m.), the interrelationships of these elements in sea water provide convenient references (Cl/Br = 660, Cl/I = 1,360,000). The ratios of Cl/Br and Cl/I in volcanic vapours varies widely between sources, as might be predicted from the volatilities of the elements at different temperatures. For reactions of type

$$2NaX_{(aq)} + H_2O_{(liq)} + SiO_{2\,(quartz)} = Na_2SiO_{3(c)} + 2HX_{(gas)}$$

Table 1 presents standard thermodynamic information at 25° (Latimer, 1952) which is useful in predicting the volatilities of the halogens in steam at high temperatures.

TABLE 1

Halogen (X)	$\Delta G°_{298}$(kcal)	$\Delta S°_{298}$(e.u.)
Cl	50·7	34·6
Br	57·0	27·9
I	49·3	18·3

From the relationship $\left(\frac{d\,\Delta G}{dT}\right)_p = -\Delta S$ it can be seen that at high temperatures (over about 800°C) the ease of hydrolysis decreases in the order chlorine, bromine, iodine. From 800°C to about 100°C the decreasing order is chlorine, iodine, bromine. Below about 100°C iodine is the most easily hydrolysed halogen, but at this low temperature the amount of hydrolysis is negligible and the differences between halogens is not great.

These are approximations in that ΔS values for the reactions have been assumed constant over the temperature range, but it is not likely that the trends will be changed by a more complete treatment (Ellis and Fyfe, 1957).

In high-temperature, low-pressure, volcanic gases the Cl/Br ratios can be expected to be high, which is as Naboko (1959) found in his survey of analyses of high temperature volcanic gases and sublimates. Bromine and iodine therefore become concentrated relative to chlorine in the crystallising igneous rocks.

COLLECTION AND ANALYSIS

The samples were collected in either glass or polythene bottles but there were no differences in the halide contents found between samples duplicated with each type of container. An earlier method using glass bottles lined with a thin film of polyvinyl acetate was unsatisfactory, as both bromine and iodine were absorbed by the liner.

The basic methods of analysis used for bromide and iodide were as described in A.S.T.M. Method Specification D1246–55 (1957).

As the iodide concentrations were low, several results were checked by an independent method which is described below and is satisfactory for concentrations over about 0·1 p.p.m. iodide.

To 100 ml of water sample add 5 cc's of phosphoric acid, sp. g. 1·75, and 1 ml of 10% hydrogen peroxide. Extract three times with 5 ml portions of carbon tetrachloride to remove molecular iodine. Shake the separated organic layer with 10 ml of an approximate 0·01M sulphur dioxide solution in N/20 HCl. Discard the organic phase and boil the acid solution to remove sulphur dioxide and carbon tetrachloride. Readjust the volume to exactly 10 cc's. Obtain the iodide concentration spectrophotometrically by means of the iodide u.v. absorption band ($\lambda_{max} = 226\ m\mu$, and $\log \xi_{max} = 4{\cdot}13$).

Table 2 compares results for iodide on several water samples by the method described, and by the A.S.T.M. method.

TABLE 2

Spring or Bore	p.p.m. I′ by Spectra	p.p.m. I′ by A.S.T.M.
Tarawera	2·5	2·6
Orakei Korako, Pudding Basin Geyser	0·2	0·4
Hole 18, Wairakei	0·2	0·3
„ 44 „	0·3	0·3
„ 49 „	0·3	0·3
„ 60 „	0·2	0·2

RESULTS

Table 3 gives the results of chloride, bromide, and iodide analyses for spring waters from many of the thermal areas in New Zealand. The spring numbers allocated by the New Zealand Geological Survey include sheet numbers of New Zealand Map Series 86. For many springs, numbers have yet to be allocated.

Concentrations below 0·2 p.p.m. bromide or iodide were not considered reliable and asterisks indicate values less than this concentration. Double asterisks on ratios indicate that they are equal to or greater than the figure reported.

Table 4 contains a selection of values for chloride, bromide, and iodide concentrations in waters collected from drillholes at Wairakei, Waiotapu, and Kawerau. The concentrations are for waters separated from the mixed steam/water discharges at atmospheric pressure. Samples collected from the pipelines at higher pressures have been corrected to bring the ion concentrations up to those that would exist at atmospheric pressure (Ellis and Wilson, 1960).

DISCUSSION

Spring Waters

The environments of many of the springs of the central North Island listed in Table 3 have been described by Grange (1937). Areas which have been described in detail are: Tokaanu, by Healy (1942); Wairakei, by Thompson (1957) and Grange (1955); Waiotapu, by Lloyd (1959); South Island springs, by Collins (1953); Ngawha, by Fleming (1945); White Island, by Hamilton and Baumgart (1959).

BROMIDES

Figure 1 shows the distribution of chloride relative to bromide for the various springs. Reference lines have been drawn for Cl/Br ratios of 660

(as for sea water) and of 880 (as for Wairakei bore waters). Most points for springs in the central North Island Quaternary rhyolite pumice, ignimbrite region stretching from south of Lake Taupo to the Bay of Plenty, fall between or close to these reference lines. As with previous results for alkali metals (Ellis and Wilson, 1960; Golding and Speer, 1961) the Cl/Br ratios in the waters of this area reflect the essentially similar geological pattern. The similar compositions imply geological processes, operating beneath the region, which are uniform with respect to temperature and chemical composition of magma. Local surface intrusions of basalt or andesite relatively close to the hot spring areas (as at Waiotapu) do little to modify the water characteristics, and evidently play little part in the mechanism of hot spring genesis.

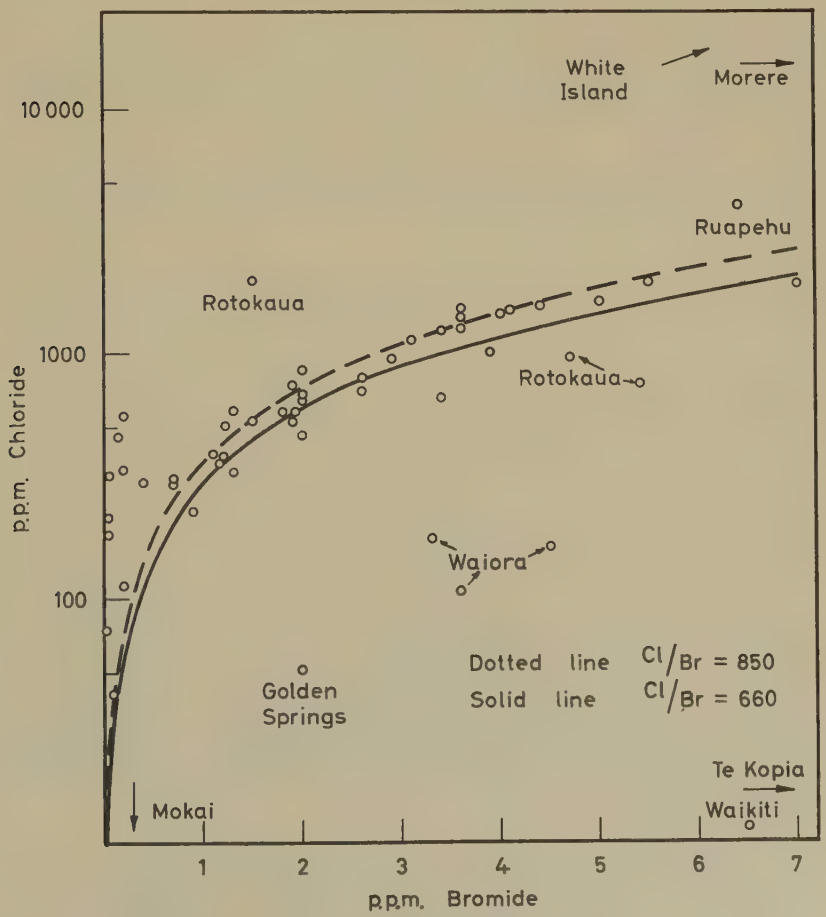

FIG. 1—Distribution of bromide relative to chloride in spring waters.

TABLE 3—New Zealand Thermal Waters

Area	Spring	Date	pH	Cl′	Br′	I′	Cl/Br	Cl/I
NORTH ISLAND								
Ruapehu	Crater Lake	12/59	1·4	3977	5·8	1·2	1550	12000
Tokaanu	Bore 1 (D.S.I.R.)	3/60	8·7	1633	5·0	<0·2	740	>30000
,,	Bath's source (1, Healy (1942))	6/55	6·9	1239	3·4	0·4	820	11000
,,	Former Geyser, N. side of road (14, Healy (1942))	6/55	7·2	1956	5·5	0·6	800	12000
Waihi	opposite Church (Healy 1942)	3/60	6·7	462	<0·2	1·5	>5000	1100
Taupo Spa	A.C. Baths N/94/7/92	6/55	5·7	16	<0·2	0·3	>200	200
,, ,,	Paddle Wheel N94/7/56	1/54	8·0	1592	4·4	0·8	820	7000
,, ,,	Eunice Geyser N94/7/41	1/54	7·8	1400	3·6	0·4	880	13000
Taupo	Spring by lake shore	5/51	3·1	123	<0·2	1·3	>1400	340
,,	Kathleen Spring	7/51	7·5	41	<0·2	0·7	>500	500
Waiora	Devil's Eyeglass N94/4//491	5/51	3·2	659	3·4	1·2	440	2000
,,	Heavenly Twins N94/4/418	3/60	2·5	163	4·6	2·8	80	210
Wairakei	Champagne Cauldron N94/4/97	5/51	8·0	1770	4·0	0·7	1000	9000
,,	Eagle's Nest Geyser N94/4/131	11/54	8·4	1388	3·9	0·2	800	25000
,,	Haematite Geyser N94/4/85	11/54	8·7	1416	3·9	0·6	820	8000
,,	Devil's Inkpot Geyser N94/4/217	11/54	8·9	1264	3·6	0·6	800	8000
Rotokaua	Black Geyser N94/5/65	3/60	2·6	963	4·6	0·6	470	6000
,,	Spring N94/5/35	3/60	5·7	1988	1·5	0·6	2990	12000
,,	Spring N94/5/96	3/60	2·9	740	5·4	3·5	310	760
,,	Spring N94/5/8	3/60	3·3	1620	3·6	1·7	1020	3400
Mokai Geyser Valley	Southern End of Valley	3/60	6·5	7·8	0·3	<0·2	60	>150
Ngatimariki	Large Pool	3/60	7·3	462	2·0	0·8	520	2000
Ohaki	Large Pool	3/60	8·8	1080	3·0	0·6	810	6000
Orakei Korako	"Pudding Basin" N85/8/6	3/60	—	401	0·8	0·3	1130	5000
,, ,,	Waipapa Geyser N85/8/24	6/55	9·0	434	<0·2	0·7	>4900	2200
,, ,,	Spring above bog on route to Wainui Geyser	11/54	9·0	314	<0·2	1·6	>3500	930
,, ,,	Kahurangi Cauldron Overflow	11/54	7·9	274	0·6	0·2	1000	5000
,, ,,	Dreadnought Geyser	11/54	6·5	308	1·0	0·3	700	4000

" "	Diamond Geyser	11/54	8·7	310	<0·2	0·5	>4000	2300
" "	Champagne Pool	11/54	8·7	388	1·1	<0·2	800	>7000
" "	Pool below Wainui Geyser	11/54	9·0	306	<0·2	0·4	>3000	3000
Waiotapu	Champagne Pool N85/6/64	6/55	6·5	1880	6·8	0·4	620	17000
"	Postmistress Pool N85/6/20	6/55	8·8	683	2·0	0·8	770	3000
"	Lake Ngahoro Spring N85/6/97	6/55	6·6	1785	6·0	—	670	—
Golden Springs	Upper Pool	3/60	6·6	50	2·0	0·5	60	350
Waimangu	Echo Lake	6/55	3·6	756	2·5	0·4	680	7000
"	Iodine Spring	6/55	9·0	863	2·0	0·7	970	4500
"	Inferno Crater	11/54	2·6	947	2·9	1·1	740	3100
Whakarewarewa	Pohutu Geyser	11/54	8·7	600	2·2	0·6	620	3500
"	Te Horo	7/55	9·2	592	1·3	0·3	1030	7000
"	Ngararatuatara	7/55	9·2	535	1·5	0·3	800	6000
Rotorua	Golf Links Bore	7/55	8·3	735	1·8	0·3	920	9000
"	Rachel Spring	7/55	9·1	558	0·2	0·2	6000	10000
"	High School Bore	7/55	—	338	0·2	0·3	4000	4000
Mokoia Island	Hinemoa's Pool	3/60	6·8	67	<0·2	0·2	>800	1200
Lake Rotoiti	Lake Shore	3/60	9·2	234	0·9	0·8	590	4200
Tikitere	Cookson's Bore	3/60	8·7	320	<0·2	1·3	>4000	880
Onepu	Spring on Tarawera River Bank	3/60	8·7	355	1·2	0·7	670	1800
"	By Lake Rotoitipaku – Pool to E. of Lake	3/60	7·7	521	1·5	0·8	780	2300
Awakeri	Pukaahu Spring	12/58	8·6	36	0·2	0·5	>400	250
White Island	Head Stream Draining 7 Dwarfs	11/49	0·2	62700	29	2·4	4900	95000
Ngawha	Venus Bath	11/59	6·1	186	<0·2	0·4	>2000	1700
"	Milky Way	11/59	6·7	228	<0·2	0·8	>3000	1000
Waiwera	Domain	5/54	7·2	1121	3·3	1·2	770	3300
Te Aroha	CO_2 Geyser	12/58	7·8	518	1·6	0·6	730	3100
Morere	Spring feeding Baths 1 and 2	1/59	6·7	16000	80	25	450	2300
Tarawera, Napier-Taupo Road	Warm Spring	1/59	8·1	660	1·9	2·5	780	940
SOUTH ISLAND								
Hanmer	Tank in Grounds	1/57	—	501	1·4	0·8	800	1400
Maruia	Bath Supply	1·58	—	142	1·0	0·2	320	2500
Banks Peninsula	Lyttelton Tunnel	1/55	7·1	513	1·9	<0·2	610	>9000
" "	Cass Bay	1/55	7·6	264	0·4	<0·2	1500	>>5000
" "	"Ferrymeade"	1/55	7·5	290	1·4	<0·2	460	>5000

TABLE 4—Waters from Drillholes

Area	Bore No.	Date	pH	Cl′	Br′	I′	Cl′/Br′	Cl′/I′
Wairakei	4/1	8/59	8·5	2112	5·6	<0·2	850	>40000
,,	4/2	10/56	8·5	2215	6·2	0·3	800	30000
,,	9	12/56	—	1889	4·8	0·4	890	17000
,,	13	8/59	—	2023	5·2	1·2	880	6000
,,	18	8/59	8·2	2265	5·9	0·3	870	30000
,,	19	9/56	8·5	2474	5·5	0·3	1010	30000
,,	20	8/59	8·4	2208	5·5	0·4	900	20000
,,	23	8/59	8·3	2005	5·0	0·3	900	25000
,,	26	8/59	8·2	2201	5·5	—	900	—
,,	27	8/59	8·5	2272	5·9	0·6	870	14000
,,	28	8/59	8·4	2180	5·0	0·6	980	10000
,,	31	8/59	8·2	2119	5·2	0·7	920	11000
,,	38	9/56	8·2	2150	5·8	0·7	840	11000
,,	39	9/56	8·5	2130	5·4	0·4	890	15000
,,	40	10/56	8·2	1900	5·2	0·4	820	17000
,,	41	8/59	8·5	2126	5·9	0·3	810	25000
,,	43	10/56	8·2	2050	4·8	—	960	—
,,	44	8/59	8·6	2258	6·0	0·3	850	27000
,,	46	8/59	8·6	2237	5·7	0·4	880	20000
,,	49	8/59	8·5	2212	5·4	0·3	920	26000
,,	53	8/59	8·1	2219	5·6	0·3	890	26000
,,	55	8/59	8·5	2303	6·0	0·2	870	40000
,,	56	8/59	8·5	2335	6·3	0·2	840	40000
,,	58	8/59	8·6	2122	5·8	0·3	820	25000
,,	60	8/59	8·4	2143	5·4	0·2	890	40000
,,	203	8/59	7·8	2041	5·0	0·3	920	25000
Waiotapu	4	7/59	—	1997	6·1	0·5	740	14300
,,	6	1/59	8·8	1450	4·7	0·2	690	30000
,,	7	7/59	8·7	1144	3·2	0·6	800	70000
Kawerau M.O.W. Bore 1		7/54	7·5	1095	4·2	0·9	590	4400

TABLE 5—Analytical Results for White Island Waters

Spring	Date	Cl	Br	I	Cl/Br	Cl/I
White Island Lake	1911	48210	34	—	3200	—
Pool, 1933 Crater	1939	61840	40	6	3500	40000
Pool, terrace, east side Island	1949	516	0·2	0·2	5000	8000
Drain from 7 Dwarfs fumaroles	1949	62600	28·9	3·9	4900	57000
Middle of 1933 Crater	1949	81700	351	14·3	520	20300
Green Pool near Noisy Nellie	1949	44100	135	7·8	740	20000
Spring on Beach	1949	4970	10·0	0·44	1120	40000
Main Stream	1949	660	1·2	0·05	1200	50000

Some earlier analytical results for White Island waters are presented in Table 5. The first two analyses are taken from Wilson (1959) and the remainder are unpublished Dominion Laboratory analyses by Miss A. Camden-Cooke.

Waters from White Island and Ruapehu, both recently active andesite volcanic centres, tend to have high Cl/Br ratios. The ratios at White Island are not constant with time, e.g., the 1939 and 1949 results for the 1933 Crater pool. It is probable that pools in this environment are heated by relatively low-pressure, high-temperature, volcanic steam. As shown above, the bromine concentration of this steam would be low relative to chlorine. The same explanation probably applies to Ngawha.

There is also the possibility at White Island of sea water coming into contact with hot rock at some depth. The resultant steam would have a high Cl/Br ratio. The alkali halides not carried by the steam and left in the hot rock would be high in bromine and iodine, which could at a later, cooler, stage be leached and carried to the surface.

Similar high Cl/Br results for recent volcanic areas were quoted by Naboko (1959). From a series of analyses of sublimates and condensates from high-temperature volcanic gases, he found that Cl/Br ratios tended to be high. The highest ratios were for sublimates from freshly erupted basalt flows, for which ratios up to 80,000 were quoted.

With respect to halogen chemistry there are, therefore, in the North Island areas of Quaternary volcanism, two main types of thermal water systems. The first type is heated directly by high-temperature, low-pressure, volcanic steam containing a relatively low bromine content.

The second type of spring contains neutral waters high in chloride, associated with the rhyolite pumice and ignimbrite region. Here, the chemicals present are considered to be brought into a deep-circulating ground water system when it mixes with a high-density aqueous magmatic fluid associated with a crystallising rhyolite magma several kilometres beneath the area. The system remains under high pressure and steam is not lost until the temperatures are below those where appreciable hydrolysis of halogens occur. In this system separation of chlorine and bromine by differential volatility is not effective, but at any temperature there is a definite distribution of chlorine and bromine between the crystallising rock minerals and the aqueous magma. The Cl/Br ratios defined in this deep mixing zone will be similar to those in the surface pools.

Halogen analyses for North Island igneous rocks are now required for two reasons. Firstly, they would allow the interaction of the thermal waters with their containing rocks to be examined. Secondly, they could give valuable information on high-temperature losses of volatiles, which would be particularly useful in studies of the processes of ignimbrite emplacement.

Further details of halogen variations in springs of the central rhyolite region may be examined.

Waiora Valley is an example of a secondary hydrothermal area heated mainly by steam separating from hot chloride water at a depth of about 1,000 ft. The pools are acid sulphate-chloride type, the acidity arising from hydrogen sulphide oxidation and hydrolysis of sulphur by hot water. The relatively low Cl/Br ratios probably result from leaching of rhyolitic rocks containing these halogens in a similar proportion. Two pools at Rotokaua also appear to be of this type, although it seems that heating in this area is largely due to hot chloride water, and the acidity may be caused by

hydrolysis of old sulphur deposits in the area. The two pools with high Cl/Br ratios at Rotokaua do not fit this explanation and no other can be suggested. Heating by high temperature volcanic steam is unlikely.

The results from Orakei Korako are variable, although the major chemical constituents of springs in this area are similar in quantity and ratios. Several geyser waters are low in bromine. It is possible that bromine is lost when the geyser blows, but as with its loss from sea water the volatile compound of the element is not known. The fact that iodine is retained in geyser waters may be due to aerial oxidation to iodate.

In the rhyolite region there are a few pools with high Cl/Br ratios, such as at Waihi and Taupo Lake shore. These pools are of low output and usually low ionic strength. It is possible that bromide and iodide may be added or extracted by algal growth in the pool environment. This is not a satisfactory explanation of the low bromide values for the Rotorua waters and Tikitere, nor is heating by low-pressure, high-temperature, volcanic steam likely in these areas.

For low-temperature springs relatively low in chloride, such as Golden Springs and Lake Rotoiti, the low Cl/Br ratios could be due either to a relatively high contribution of bromine from meteoric waters or leaching of sediments at shallow depths.

Morere and Tarawera springs in the North Island are in regions without obvious volcanic associations, the former being in an area of Tertiary sediments and the latter in a greywacke area. The Cl/Br ratio for Tarawera is similar to the average for the rhyolite areas, but the Morere spring is relatively high in bromide as is to be expected from its association with sedimentary rocks.

In the South Island, the springs at Banks Peninsula, in an area of Tertiary basic igneous rocks, are only a few degrees hotter than the average air temperature. They are possibly meteoric waters with the halogen contents controlled by sea moisture, rock leaching, and, in the Cass Bay spring, extraction by algal growth. This water was covered with a green slime when collected.

The other two South Island warm springs lie along the Great Alpine Fault (Wellman and Willett, 1942) in greywacke and schist country with no known surface volcanic rocks in the area. A low Cl/Br ratio could be predicted if the halogens were derived from leaching of local rocks but only the Maruia value is in agreement. No obvious distinction between waters of volcanic and metamorphic origin can be made on the basis of their chloride and bromide contents.

IODIDES

Figure 2 shows the distribution of iodide relative to chloride concentrations. Only a few general remarks are warranted.

Many of the values for springs in North Island volcanic areas are in the 0·3 p.p.m. to 1·0 p.p.m. range and show little relationship to chloride concentrations.

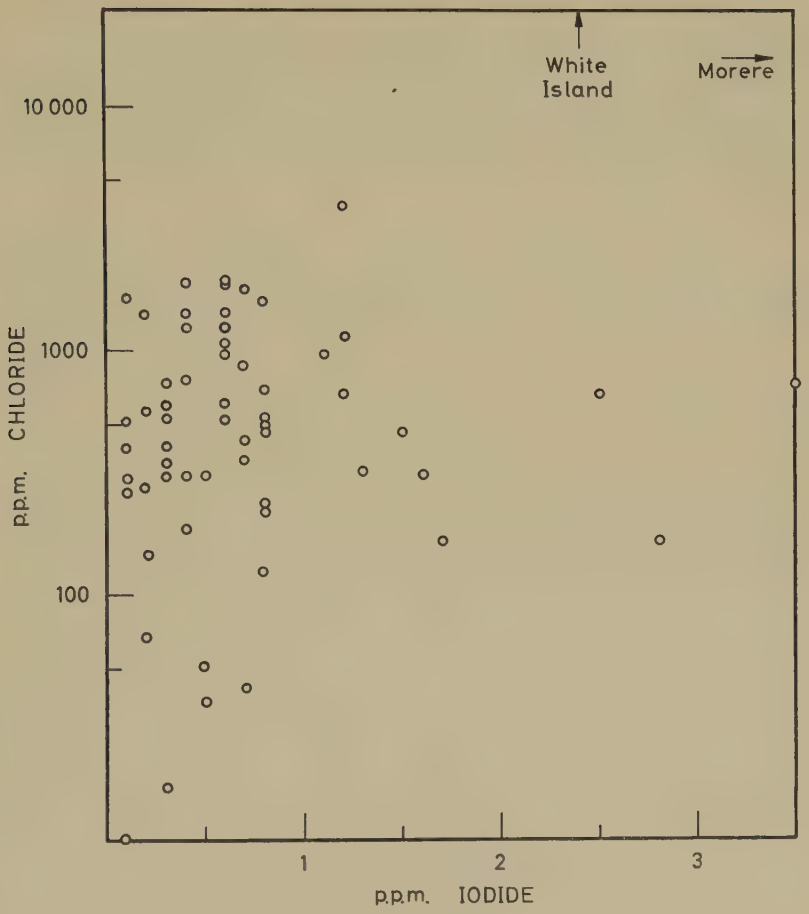

FIG. 2—Distribution of iodide relative to chloride in spring waters.

For the neutral, high-chloride, high-output, springs in areas such as Tokaanu, Waiotapu, Whakarewarewa, and particularly for the excellent samples given by Wairakei bores, Cl/I ratios of the order of 20,000 are common. Such ratios may represent the relationship of these elements in the high-temperature magmatic water phase that becomes diluted with convecting meteoric water. The amount of iodine carried right through the convection cycle with the meteoric water will be low, except where local surface waters carry a high halogen content. This could occur where the atmosphere has a high content of marine volatiles, which does not apply in the central North Island.

The concentration of iodine in many igneous rocks is of the order 0·1 p.p.m. to 1 p.p.m. (Correns, 1956). It is therefore possible that the

small amounts of iodide found in many of the minor springs of low outflow simply reflect partial leaching of the country rocks by the water.

High iodide values appear in springs where leaching of sedimentary material is likely, e.g., 25 p.p.m. of iodide at Morere. The values from Waiwera, Waihi, and Taupo Lake shore may result from a similar process. The concentration of 2·5 p.p.m. iodide in the Tarawera hot spring on the Napier-Taupo Road may reflect a high concentration of iodide in the country greywacke.

The high iodide concentrations at Tikitere, Inferno Crater, Rotokaua, and Waiora Valley seem unlikely to be derived from leaching of local igneous rock. The values imply preferential transfer of iodine by a steam phase heating the pools.

Waters from Drillholes

WAIRAKEI

Maps of the Wairakei field showing the numbering of drillholes were given in Ellis and Wilson (1960). The average Cl/Br ratio is 863 for the eastern part of the field (drillholes 4/1, 4/2, 9, 23, 38, 39, 40, 41, 43, 53, 58, 60), while for the western holes the average is 890. For the natural springs at Wairakei the average Cl/Br ratio is 855. The comparable Cl/I ratios for these sources are, respectively, 25,000, 24,000, and 12,500.

The differences in Cl/Br ratios between the three sets of samples is of little significance, and the results add further support to the conclusion (Ellis and Wilson, 1960) that all the waters in the Wairakei area have a common source. The relatively higher iodide concentrations in the spring waters are probably due to leaching of surface sedimentary layers containing organic material.

As shown by Ellis and Wilson (1960) there has not been any major change with time in the chemical characteristics of waters from drillholes in the area. In the last five years the chloride and alkali concentrations have remained approximately constant.

The bromide and iodide results confirm the constant composition of the supply. For the Wairakei drillholes the average Cl/Br ratios for the 1956 set of samples is 888, with Cl/I equal to 20,000. In 1959 the comparable ratios were 887 and 25,000 respectively. Later results (1961) show very similar ratios.

The variations in Cl/I ratio over the Wairakei bore field cannot be explained simply. The higher iodide concentrations may be due in some cases (shallow holes 9 and 13) to leaching of pumice horizons containing organic material, but this is unlikely for deep holes. Hole 27 is thought to tap a fissure zone through the ignimbrite, and possibly the result for this bore shows the iodide concentration in the water as it enters the permeable breccias from below. Adsorption by clay minerals or leaching of organic materials, may raise or lower the concentration from this value.

WAIOTAPU

The results for waters from drillholes may be compared with those for natural spring waters. From the Cl/Br ratios the waters tapped by the holes correspond more closely to the water emerging at Postmistress Pool, rather than to the two other major springs analysed. Comparisons between iodide results probably have little significance, as in the Wairakei examples.

RESULTS FROM OTHER COUNTRIES

Table 6 gives a selection of chloride, bromide, and iodide results from some thermal areas in the circum-Pacific belt of volcanic centres. Results from Iceland (Bodvarsson, pers. comm.) are also included.

From Iceland the Krisuvik example is a bore near the coast, and the bromide and iodide may be derived from sea water. The Hveragerdi bore draws from a deep hot water system at a temperature around 200°C. The mechanism of this hydrothermal system through basaltic country rock may resemble that of Wairakei, New Zealand.

The low bromide concentrations for the Nevada and Wyoming springs are surprising. Yellowstone Park is in a rhyolite area and many of the chemical characteristics of the springs resemble those of springs in the central rhyolite region of the North Island, New Zealand. Iodide concentrations are similar to those of New Zealand springs.

Koga (1959) reported mean values for halogens in springs around Beppu, Japan. Kannawa and Kamegawa were considered to be associated with recent volcanism. In the Metropolitan region, where the Cl/Br ratio is the same as for local rocks (434), simple rock leaching is indicated.

According to Piip (1937) the high chloride springs of Kamchatka are mainly associated with recent effusive formations of andesite and basalt, and his results showed that Cl/Br ratios were similar within each group of springs. The variations between localities are greater than exist between major chloride springs in the North Island of New Zealand.

ORIGIN OF HALIDES

As reviewed by Mason (1958) the amounts of chloride and bromide in the sea are much greater than could be derived from the weathering of rocks throughout geological time. These elements may have been accumulated gradually from the outputs of volcanic and hot spring centres. As an alternative, or in addition, halogens may have been present in a primordial ocean. The results presented and reviewed in this paper show that, in general, volcanic vapours and hot spring waters have a Cl/Br ratio greater than that of present day sea water. As volcanic activity has undoubtedly contributed to the halide content of the oceans throughout geological history, it seems that it is necessary to assume a primordial sea relatively rich in bromide to account for the present Cl/Br ratio after the addition of low-bromide volcanic fluids. It seems less valid to assume that the halogen composition of volcanic vapours has changed through geological time.

TABLE 6

Area	Spring	(Ref.)	Temp. °c	pH	Cl′	Br′	I′	Cl/Br	Cl/I
Iceland	Krisuvik, Bore	Bodvarsson	100	9·3	735	4·8	0·1	340	30000
	Hveragerdi, Bore	„	100	9·4	152	0·5	0·0	700	—
Nevada	Steamboat Springs	White (1957)	89	7·9	865	0·2	0·1	9700	30000
Wyoming	Yellowstone Park, Norris basin	„	84	7·45	744	0·1	<0·1	17000	>30000
„	Upper Yellowstone Park, Old Faithful Geyser	Gooch & Whitfield (1888)	—	—	440	3·5	—	280	—
Japan	Beppu Springs	Koga (1959)							
	Myoban region average	„	57	3·6	11·4	0·037	0·013	695	3140
	Kannawa region average	„	85	3·7	1695	4·49	1·111	850	5370
	Kamegawa region average	„	58	7·3	514	1·69	0·153	684	11900
	Hotta Kwankaiji region average	„	53	6·9	22·7	0·083	0·027	615	3000
	Metropolitan region average	„	56	6·9	191	0·89	0·144	446	4770
Kamchatka	Pavzhetskie, Geyser No. 1	Piip (1937)	97	6·7	1713	2·5	—	1550	—
„	„ Parayzii, No. 1	Ivanov (1958)	100	8·4	1684	3·2	—	1190	—
„	Nizhne – Golyginskie	Piip (1937)	70	—	1195	1·6	—	1700	—
„	Nizhne – Paratunskie, Zavoiko	„	81	—	167	0·4	—	950	—
„	Bolshbannye	„	100	—	112	0·3	—	850	—
„	Nachikinskie	„	70	—	145	0·2	—	1600	—
„	Nalachevskie, No. 1	„	73	—	1595	6·0	1·0	600	5700
„	Kraevadcheskie, No. 1	„	58	—	3080	8·0	2·0	865	5500
„	Pushchinskie, Group F	„	42	—	1511	25·2	—	135	—
„	Uzonskie, Lake No. 4	„	57	—	1394	6·0	—	520	—
„	„ Black source	Ivanov (1958)	—	2·3	13	0·1	—	300	—
„	Geysernye, Velikan Geyser	„	99	8·7	859	1·3	—	1500	—
Kuril Islands, Kunashir Island									
	Glavnyi, Hot field	Ivanov (1958)	100	8·1	2355	3·4	0·2	1550	40000
	Lower Mendeleevskie, Osnovnoi	„	91	1·7	983	0·3	—	7400	—

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APPLICATION OF THE ZELENY SEDIMENTATION TEST TO NEW ZEALAND WHEAT CROPS

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Summary

The Zeleny sedimentation test has been used successfully for three seasons as a means of classifying commercial lines of Arawa wheat with respect to baking quality. For Aotea, the variety now predominant, the test has failed to give a satisfactory prediction of baking quality, although failure of the sedimentation test itself has not been demonstrated; in 1959 the range of quality was narrow and in 1960 there was an anomalous milling characteristic. The test has proved successful in two seasons for the varieties Cross 7 and Hilgendorf. The relation between sedimentation score and baking score has been shown to vary with variety and year but to be substantially constant for a variety within a harvest season.

Introduction

The quality of a flour for baking bread can best be judged from a baking test. However, the recent release of the wheat variety Arawa, which in some seasons had poor baking quality, made desirable the use of a rapid method for testing individual lines of wheat so that the poorer lines could be excluded from bread flour grists. The sedimentation test developed by Zeleny (1947) (Pinckney, Greenaway, and Zeleny, 1957) has been adopted in the New Zealand wheat breeding programme (Dep. Sci. Ind. Res. (N.Z.), 1952) (Copp, 1956) and it is the purpose of this paper to record the experience obtained with the test on commercial samples.

Preliminary development work was carried out on samples of Arawa from the 1958 harvest. The method was then applied to 5,200 samples submitted by merchants in the period from January to March 1959. These samples represented almost 80% of the crops harvested in 1959 and included all the commercial varieties grown in New Zealand. The standard baking test was applied to more than 600 of the samples and other determinations were made on bulks prepared from groups of samples. In view of the increased production of Aotea and the decrease of Arawa in the 1960 harvest, the large-scale use of the test was reviewed. As the 1960 harvest progressed it became apparent that the test was not a reliable guide to baking quality for Aotea and its use was discontinued.

Earlier work by Copp (1956) showed that the agreement between sedimentation score and baking score was not good when varietal differences were ignored, but that satisfactory correlations could be obtained for individual varieties. The main part of the present work is concerned with

the newer, high-yielding varieties Arawa and Aotea, and these are compared with Cross 7, the standard variety by which wheat bred in New Zealand is judged, and with Hilgendorf, the premium strong wheat of New Zealand. Other varieties included are Dreadnought, Fife-tuscan, and Yielder. The varieties have been described by McEwan (1959).

MATERIALS AND METHODS

To obtain flour for the sedimentation test 50 g wheat was passed through a laboratory grinder (Regent Maskin AB, Stockholm. Model R. 1,800 r.p.m.) at the finest setting. The resulting meal was shaken over a 90-mesh sieve for 30 sec. or an 8 xx silk for 20 sec., and the throughs taken as flour (Regent flour). The yield was of the order of 20%. The sieve shaker described plane circles of 8 cm radius at 100 revolutions per minute.

The method for the sedimentation test was similar to that of Pinckney, Greenaway, and Zeleny (1957). Three grammes of flour in a 100 ml stoppered cylinder was shaken for 5 min. with 50 ml of water-dye reagent, shaken for a further 5 min. after the addition of 25 ml of lactic acid-*iso*-propanol reagent and finally stood 5 min. before the settled volume was read in front of a fluorescent lamp. The cylinders were graduated 0 to 100 ml over a length of 155 to 160 mm. The shaker worked at 45 cycles per minute through a total angle of 60° about the horizontal. The water-dye reagent contained 4 mg of brom-phenol blue per litre. The acid reagent contained 45 ml of 85% lactic acid (diluted and refluxed) and 200 ml of *iso*-propanol per litre.

Flours for test baking (Allis-Chalmers flours) were prepared on the Allis-Chalmers experimental mill to 78% extraction. The baking formula was 125 g flour, 1·88 g yeast, 2·5 g salt and 0·94 g sugar (2·5 g sugar if necessary). The doughs were mixed 1½ min. at 78°F in a McDuffee type, water-jacketed, pin mixer, 110 r.p.m., at optimal absorption. They were fermented, with two knocks, and proofed at 78°F, with a total of 5¾ hr to the oven. Loaves were allowed to cool overnight and then scored for volume, crumb texture, and crumb colour. Volume is expressed on a scale derived from an earlier judging system, in which the metric volume is (18·5 × score + 300) ml and an average score is 18 points. Crumb texture is expressed on a 14-point scale (average about 11) and colour on an 8-point scale (average about 5). The total score is the sum of the three. Flours were baked with 0, 10, and 20 p.p.m. potassium bromate and the scores reported here are for the best (mature) loaf.

Varietal bulks were prepared from the residues of the samples sent in for Zeleny testing. For Arawa, several bulks were prepared from samples grouped according to sedimentation score. Each bulk was carefully mixed and sampled by conventional procedures.

Flours were prepared from the 1960 varietal bulks with the rolls of a Tag-Heppenstall moisture meter (Tag-roll flours) as described by Pinckney, Greenaway and Zeleny (1957).

RESULTS

Usefulness of Test in Evaluating Individual Samples

More than 1,000 samples have been subjected to both sedimentation and baking tests. The results have been analysed statistically to decide how well the baking score can be predicted from the sedimentation score of individual samples. Variation between the four main varieties, variation within each variety during the harvest season, and variation for a variety from season to season have been considered. Some of the results are presented in Table 1. Results of sedimentation and baking tests and other analyses carried out on varietal bulks are given in Table 2.

The effectiveness of the test in predicting baking scores is expressed in the last column of Table 1. A useful prediction was obtained for Arawa in 1958 and for Arawa, Cross 7, and Hilgendorf in 1959, but not for Aotea in 1959, even if the Allis-Chalmers flour was used. In 1960 the test was effective in providing useful information for Arawa and Hilgendorf and, if Allis-Chalmers flour was used, for Aotea.

Variety differences in the relation between sedimentation score and baking score, and the variation in that relation between years and within one year for particular varieties, have been examined by within- and between-group regression analysis of variance. The significance of the gain in precision in the prediction of baking score from using separate regression equations for each group has been calculated and the results may be summarised as follows:

Comparisons between Varieties

1. 1959 Regent flours of Aotea, Arawa, Cross 7, and Hilgendorf were compared. The gain in precision from using separate regression equations was significant at the 99% probability level. The only pair of varieties which could be described by one regression line was Aotea and Cross 7.

2. 1960 Allis Chalmers flours of Aotea, Arawa, and Hilgendorf were compared. The gain was significant at the 99% level. No pair of varieties could be described by one line.

3. 1960 Regent flours of Arawa and Hilgendorf were compared. There was no significant difference in the slope of the regression lines, but a significant difference in constant.

Comparisons between Years

4. Regent flours from Arawa lines of 1958, 1959, and 1960 were compared. The gain from separate equations was significant at the 99% level.

5. Hilgendorf, Regent flours of 1959 and 1960 were compared. There was no significant differences in the slope of the regression lines, although there was a significant difference in constant.

6. Aotea, Allis-Chalmers flours of 1959 and 1960 were compared. The gain was significant at the 95% level.

TABLE 1—Comparison of Sedimentation and Baking Scores

Year and Variety	Number of Samples	Sedimentation Score Mean	Sedimentation Score St. Dev.	Baking Score† Mean	Baking Score† St. Dev.	Regression Line Slope	Regression Line Const.	% Reduction in St. Dev. Using Sed. Score
Allis-Chalmers Flours								
Arawa 1960	105	34·6	7·0	32·8	2·0	0·217	25·3	32
Aotea 1959	35	31·6	6·1	34·8	2·1	0·161	29·7	10
1960 North	131	23·9	6·8	31·5	2·9	0·293	24·5	26
South	75	27·6	4·6	34·1	1·6	0·238	27·6	25
TOTAL	206	25·2	6·3	32·5	2·8	0·316	24·5	29
Hilgendorf 1960	32	34·7	7·7	35·4	2·5	0·244	26·9	33
Regent Flours								
Arawa 1958	55	31·1	8·6	29·4	5·1	0·503	13·8	48
1959a*	84	39·7	11·3	33·2	3·9	0·258	23·0	34
1959b	95	37·6	7·7	33·1	3·0	0·208	25·3	15
1959c	95	36·6	8·0	33·8	2·5	0·204	26·3	24
1959d	95	37·1	7·4	33·5	2·2	0·189	26·5	23
1959 TOTAL	369	37·7	8·6	33·4	2·9	0·216	25·3	23
1960 North	105	35·0	8·9	32·8	2·0	0·163	27·1	29
Aotea 1959a	38	27·0	8·0	34·3	2·3	0·118	31·1	7
1959b	41	25·9	6·6	34·5	1·9	0·112	31·6	7
TOTAL	79	26·4	7·3	34·4	2·1	0·114	31·4	8
1960 North	131	17·1	6·6	31·5	2·9	0·139	29·1	5
Cross 7 1959a	42	32·5	15·7	35·9	3·7	0·186	29·8	39
1959b	45	27·9	10·5	35·1	3·0	0·169	30·3	18
TOTAL	87	30·1	13·4	35·5	3·3	0·181	30·0	30
Hilgendorf 1959a	37	41·4	12·8	39·5	2·7	0·159	32·9	33
1959b	37	42·2	11·2	40·5	2·4	0·105	36·1	12
TOTAL	74	41·8	12·0	40·0	2·6	0·137	34·3	22
1960	32	24·3	14·2	35·4	2·5	0·135	32·1	35

*The designations a, b, c, d, refer to consecutive halves or quarters of the harvest seasons.
†All baking scores were determined on Allis-Chalmers flours.

TABLE 2—Analyses of Varietal Bulks

	Arawa					Aotea	Cross 7	Hilgf.
	e*	f	g	h	i			
1959								
Baking score	32	32	35	37	38	36	38	41
Protein (%)	9·3	9·3	10·5	11·6	12·6	10·9	11·4	12·9
Allis-Chalmers flours								
Sedimentation score	27	29	35	39	44	28	32	42
Ash (per 10^4)	48	45	44	45	45	52	47	46
Colour grade	4·3	4·0	4·5	4·9	4·9	3·9	3·6	4·8
Effectiveness of test†						10		
Regent flours								
Sedimentation score	24	29	36	42	47	26	33	47
Ash	74	73	71	70	68	106	95	91
Colour grade	8·3	8·2	8·8	9·0	9·1	11·2	10·6	12·6
Effectiveness of test			23			8	30	22
1960								
Baking score	32	32	34	35	36	33	35	38
Protein	8·6	9·1	10·2	11·2	12·0	9·0	10·1	12·0
Allis-Chalmers flours								
Sedimentation score	24	28	32	36	40	27	33	37
Ash	53	52	51	50	49	61	57	58
Colour grade	3·2	3·7	4·3	4·8	5·2	3·6	3·8	5·5
Effectiveness of test			32			29		33
Regent flours								
Sedimentation score	20	28	35	42	46	8	10	32
Ash	74	65	63	60	62	103	104	103
Colour grade	7·4	6·8	7·4	7·6	8·8	10·4	9·7	13·3
Effectiveness of test			29			5		35
Tag flours								
Sedimentation score	15	20	28	35	41	15	30	42
Ash	36	36	35	36	36	42	42	40
Colour grade	4·6	3·9	4·4	4·9	5·3	4·0	4·3	6·0

*The groupings e to i are according to sedimentation score of Regent flour for each line. e, below 24; f, 24 to 30; g, 31 to 37; h, 38 to 44; i, over 44.

†Data of last column of Table 1, repeated to facilitate comparison.

Comparisons within One Season

7. Arawa: 1959 Regent flours. There was no significant variation between the consecutive quarters of the harvest season. Comparisons of the first and last quarters showed a gain which was just significant at the 95% level. This may be a reflection of the tendency of the earliest samples from each harvest to be anomalous.

8. Aotea, Cross 7, Hilgendorf: 1959 Regent flours. There was no significant difference in any variety between the earlier and later parts of the season.

9. Aotea: 1960 Allis-Chalmers flours. Samples from the northern and southern parts of the crop area showed no significant difference in the slope of the regression lines, but there was a significant difference in constant.

There were, of course, highly significant differences to be found between groups in mean sedimentation scores and baking scores. These may be tested directly from the data of Table 1.

Effect of Milling Method

The aim has been to produce on a small scale a flour which in the sedimentation test would give results showing a close relationship with those obtained with 78% extraction flour from the Allis-Chalmers experimental mill. The Regent mill was used because grinding with the rolls of the Tag-Heppenstall meter as recommended by Pinckney, Greenaway, and Zeleny (1957) was rather slow for the large-scale testing service being considered. The use of sieves finer than 90 mesh did not affect the sedimentation score for Arawa samples but gave some improvement for other varieties.

A comparison of the bran contents of the three types of flour milled from the varietal bulks may be obtained from the ash and colour grade figures of Table 2. The ash contents of the Regent flours are far above the 0·6% specified by Zeleny; the results obtained with the sedimentation test on Regent and Allis-Chalmers flours are, however, in reasonable agreement for Arawa. Sedimentation scores for Allis-Chalmers flours were considerably higher than those obtained for Regent flours of the same lines of Aotea and Hilgendorf in 1960 (Table 1). In spite of this effect, however, the accuracy of prediction of baking score for Hilgendorf was not improved by use of the Allis-Chalmers flour. In the varietal bulks this large difference between the two flours was not found in 1959 (Table 2).

Examination of the meal showed that the Regent grinder has a break action, with little reduction occurring. Samples of Arawa passed through the Regent grinder give a lower proportion of fine bran particles than do the other varieties and hence a cleaner flour, as shown by the ash and colour grade figures. This is supported by conventional milling experience with Arawa, the bran tending to remain in large chunks adhering to endosperm.

A plot of baking scores against sedimentation scores of the Regent flours for the 1959 varietal bulks (Fig. 1) shows that all the varieties except Arawa fall approximately on one line. A plot of the Allis-Chalmers flours shows a similar pattern. The two sets of sedimentation scores are similar, but for the best flours the Regent score is the higher, and for the poorer flours the Regent score is the lower. It appeared, therefore, that in 1959 the Regent grinding and sieving technique was satisfactory for the purposes of the test. In 1960, however, the Regent flour from Cross 7 and Aotea gave results in the sedimentation test much inferior to those obtained

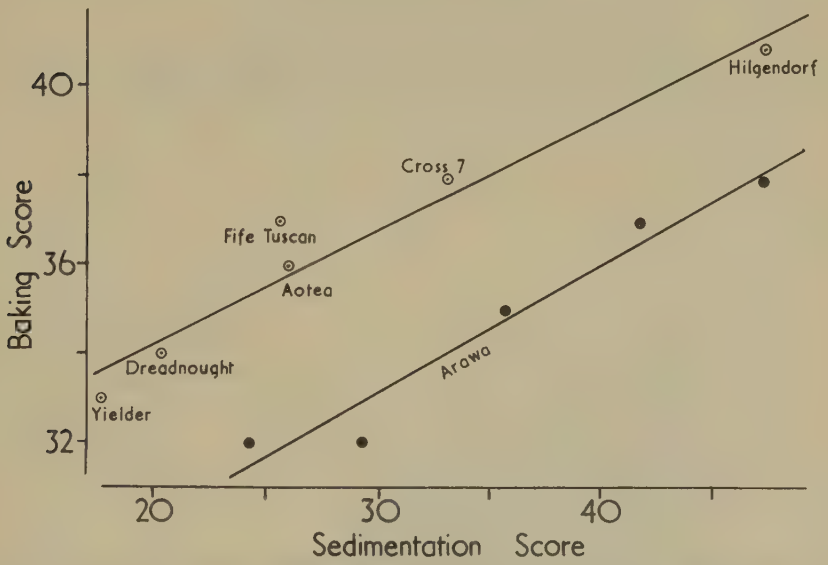

FIG. 1—Relation between baking score and sedimentation score for varietal bulks of 1959 (Regent flour).

with the Allis-Chalmers flour. There was a preponderance of low Zeleny scores obtained in 1960, as seen in the distribution diagrams (Fig. 2) for Aotea. The distribution for 1959 was more normal. The bran contents of Regent flours from the bulks for the two years were not widely different, so the explanation of this phenomenon must be sought in some other quality of the grain from the 1960 harvest.

As reported above for the testing of varietal and seasonal differences, the significance of the gain from using separate regression equations for each group has been calculated for Allis-Chalmers and Regent milling methods and may be summarised.

Aotea, 1959	.....	No significant gain.
Aotea, 1960	.....	Gain significant at 99% level.
Arawa, 1960	.....	Gain significant at 95% level.
Hilgendorf, 1960	.....	Gain significant at 95% level.

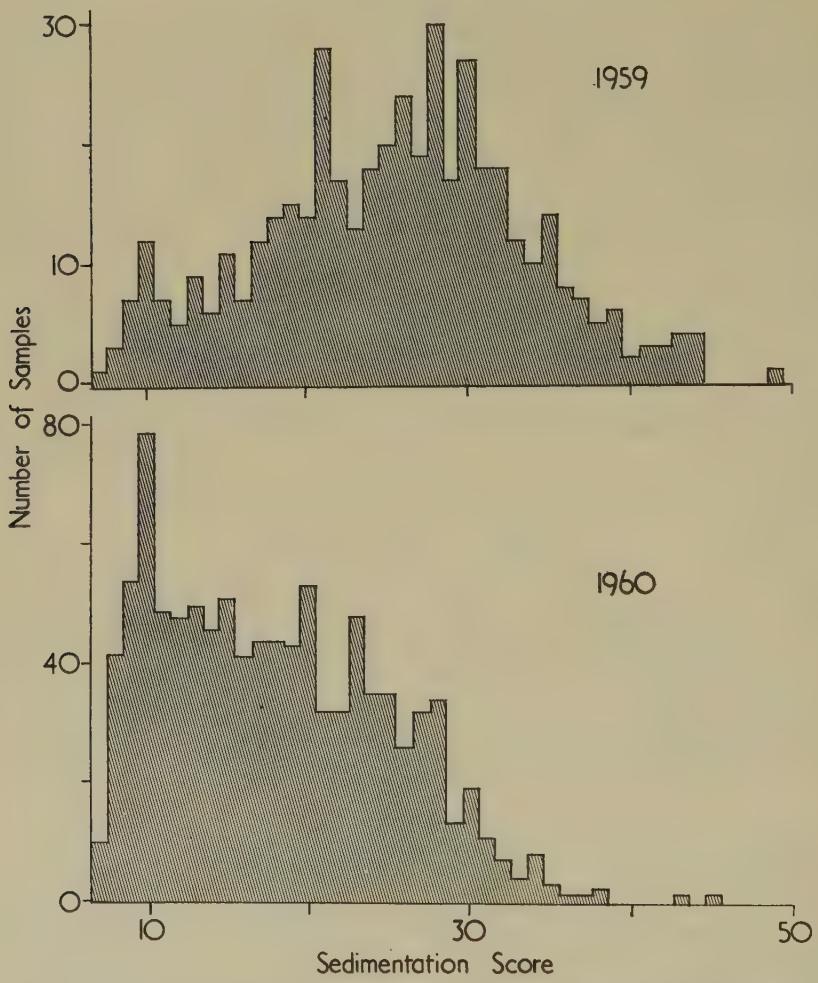

FIG. 2—Frequency diagrams for sedimentation score of Aotea samples, (Regent flour).

Concerning the Nature of the Sedimentation Test

The nature of the test and observation of the sediment suggested that the sedimenting particles are starch and protein, the protein being that fraction of the gluten complex which has a gel structure insoluble in dilute acid (Meredith, Sammons, and Frazer, 1960). Starch alone gives little sediment and the amount of starch present has little effect on either the rate of settling or the volume of the precipitate. The flocculent state of the gel is sensitive to change in ionic environment, hence the volume of sedimented protein depends on the soluble mineral content and has been found to decrease with the addition of sodium chloride (Table 3). The

effect of bran on the sediment volume may be ascribed in part to the soluble ash which it introduces. It has been found, however (Table 3) that bran exerts some specific effect far more important than that due to its ion content, since the increase in soluble ash produced by the addition of bran is much less than the amount of sodium chloride required to lower the sediment volume to the same extent. The lipid content of the flour also has an effect, which is on the rate of settling rather than on the volume (Table 3) but the effect of this factor in flour-to-flour variations has not been studied. The state of subdivision of the flour particles has a marked influence on the sediment volume, as realised by all previous workers, and additional illustration of this point is unnecessary.

TABLE 3—Effects of Salt, Bran and Lipid on Sedimentation Volume

	NaCl Added (g/100 g Flour)	Sediment Volume (ml)		Ash Content of Flour Solubles (g/100 g Flour)
		5 min. Settling	4 hr Settling	
Flour 1	0	50	24	0·38
	0·2	44	21	
	0·4	39	19	
	2·0	21	11	
Flour 1, with added bran	0	39	19	0·41
Flour 2	0	29	16	
Flour 2, defatted	0	20	10	
Flour 3	0	40	10	
Flour 3, defatted	0	19	18	

DISCUSSION

Usefulness of Test

In considering how well the baking score can be predicted from the sedimentation score for individual samples we are concerned in each instance with the slope and constant of the regression line and the scatter of the points about the regression line. We must at the start make the distinction that good correlation does not necessarily imply satisfactory information for prediction of baking score.

The results from the varietal bulks of 1959 showed that Arawa gave a regression line different in constant from that determined by the other varieties, which all approximated closely to a single line. The lines calculated for the individual samples showed roughly the same pattern, the line for Arawa being distinct from those for the other three main varieties. Statistical analysis showed that Cross 7 and Aotea in 1959 could be described by one regression line but that no other pair of varieties could. This confirms findings of previous seasons at the Crop Research Division for these two varieties. The other comparisons made showed that each variety had a regression line for predicting baking score from sedimentation score which was significantly different from the others.

Consideration of the corresponding average sedimentation scores, when varieties are ranked according to baking score, shows the danger of attempting to interpret all varieties from a single regression line. Finney (1943) showed that the relation between loaf volume and protein content differs for different varieties, hence the similar finding for sedimentation score and baking score is not unexpected.

From the three comparisons made it is clear that for any one variety the regession line may vary in different years. This being so, it is natural to consider whether variation could also occur within one season. It is not easy to separate district and date of harvest as discrete factors. In 1959, the samples from halves or quarters of the harvest period were compared and no significant difference could be found between successive periods. There was, however, a barely significant difference between the first and fourth quarters for the variety Arawa. In 1960, comparison was made between samples of Aotea from the north and south parts of the crop area and no significant difference was found in slope, although there is a difference in constant. It seems then that there may be some variation in regression line during the harvest season.

To consider how well the sedimentation score can predict baking score for a particular sample we have compared the standard deviation of the regression prediction with the standard deviation of the baking score. On this basis, in 1959, the information gained by sedimentation testing of Aotea was little better than a sheer guess based on a known mean. The information was considerably better for Arawa, Cross 7, and Hilgendorf. Whether the sedimentation test, or any other test, can reasonably predict the baking score depends very largely on the range of quality. Where the range is narrow no test can give much more information than a guess from the known mean. It is suggested that the failure of the sedimentation test on Aotea in 1959 was due to the narrowness of the distribution of baking score. A study of the distributions of baking scores for previous years has shown that while a relatively narrow distribution is possible for any variety in any year, a distribution as narrow as that for Aotea in 1959 is only likely to occur occasionally in any variety. The Arawa samples of 1960 also had a narrow distribution of baking quality and it was because of the better fit of the sedimentation test when applied to Arawa rather than to the other varieties that the test was in this case effective. We are unable to suggest any particular conditions which would give rise to a narrow range of quality.

Comparisons of the correlations for Aotea of 1959 using either Regent or Allis-Chalmers milled flours for the sedimentation test showed no significant difference in either the regression lines or the scatter of results. The method of milling did not, therefore, contribute to the uselessness of the test for Aotea in 1959.

Milling Method

Published work on the Zeleny sedimentation test has emphasised the need for reproducible milling conditions since extraction rate and granularity seriously affect the results (Schaeffer 1957).

As already stated, the reason for using the Regent grinder rather than the technique described by Zeleny was to simplify the method further and to reduce processing time, since the handling of up to 400 samples a day by a small team was envisaged. It was due to the expected preponderance of the variety Arawa in the crop that large-scale testing was proposed. This variety has a wide seasonal range of quality and peculiar milling characteristics. It was, perhaps, unfortunate that the development work in 1958 was confined entirely to Arawa, for which the Regent milling method is satisfactory.

In 1959 the technique described, using the Regent laboratory grinder in a single pass and a single sieving, yielded flours from the varietal bulks giving sedimentation scores similar to those of flours obtained from the Allis-Chalmers mill, and the small differences found followed a definite pattern. We were therefore satisfied that the Regent method of flour production for the test was satisfactory. The comparison of milling methods for the Aotea samples of 1959 was on material with so little spread of baking score that it is not surprising that no difference was found.

Our experience with the 1960 varietal bulks, however, was that whilst the Regent technique was again satisfactory for the varieties Arawa and Hilgendorf, it failed to give a satisfactory flour for the testing of Aotea and Cross 7. Considering the 1960 individual samples, for the Regent flours the improvement in information over a guess from the known mean through using the sedimentation score was considerable for Arawa and Hilgendorf but was negligible for Aotea. On the other hand, when the Allis-Chalmers flours were considered, the improvement in information was considerable for Aotea. Thus in 1960 it could be said that it was the method of milling to obtain the flour sample that was unsatisfactory for Aotea, and that results from the sedimentation test itself were satisfactory.

In 1960 the varieties Aotea and Cross 7 had some anomalous characteristic, whereby the flours produced from the Regent grinder proved unsatisfactory for the test. The bran content of these flours was no higher than that of the Regent-milled flour from Hilgendorf, which was satisfactory for the test. There was therefore some other characteristic of these two varieties in 1960 which was abnormal. Even flour produced by the Tag-roll method from the 1960 bulk of Aotea gave a sedimentation score much inferior to that from the Allis-Chalmers flour and so it is probable that the test would still have failed for the 1960 Aotea crop had the method originally described been used. The mean sedimentation score for individual samples of Regent flour of Aotea in 1960 was 17·1, whereas the score for the corresponding varietal bulk was only 8, implying that the "interfering factor" was present in some of the flours in much greater amount than was required to lower the score to the minimum reading of 7.

CONCLUSIONS

It is known that the sedimentation score varies primarily with the protein content but depends also on a number of other factors. Since the value of a flour for baking depends on many variables, it is evident that any test which measured only one of such variables would be inferior to a baking

test. The sedimentation test is affected by a number of factors in common with the baking test and to that extent may be superior to determination of total protein, as claimed by Zeleny. It is therefore potentially valuable so long as a majority of the other variables are known and controlled. It has been shown that in New Zealand some unknown variables affected by varietal and seasonal differences are of great importance. Bran and ash content are important in lesser degree.

The present work has shown that a general calibration for connecting sedimentation score and baking score is valueless under N.Z. conditions. It is necessary to have separate regression equations for each variety and calibration must be repeated each season. Although the test may be valuable for some limited applications (e.g., segregation of lines within a mill), it is not suitable as a grading test for extensive use in New Zealand.

The test as we have applied it has proved unreliable in different ways in two seasons for one of our main wheat varieties, though in neither case was the sedimentation test itself demonstrated to be at fault. It has been satisfactory for Arawa, the variety for which we originally intended to use it.

So long as baking performance is the final criterion of wheat quality it is probable that no other single test will supplant a baking test. The chief weakness of the alternative tests that have been proposed is that they cannot be used to measure the effects of sprouting or bug damage, either of which may completely override protein quality in importance for grist selection. An aim of the Wheat Research Institute is to develop a simple baking test that could be applied as a routine to several hundred wheat samples a day. Others are working along the same lines (Meppelink *et al.* 1959, Shellenberger *et al.* 1958).

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THE GROUND WATERS OF THE WAIKANAE–PARAPARAUMU REGION

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Summary

A number of wells have been examined in the Paraparaumu-Waikanae region and water from 20 wells analysed. Analyses show that there are three distinct provenances of the well waters:

(a) South of the Waikanae River, with water of low pH, high salinity and hardness, containing iron but very low in sulphate. Of rain water origin.

(b) Immediately adjacent to the river. Except for low pH, analyses agrees with that of river water. Of river origin.

(c) North of the river; low pH, but oxidised, containing nitrate and sulphate. Mixture of river and rain water.

Introduction

The population of permanent residents in the area north of Paekakariki to the Waikanae River and for several miles beyond has increased considerably in recent years and is augmented during the summer by a very large population in motor camps and seaside cottages. For water, this community depends upon tanks filled by rain collected off roofs, and supplies from shallow wells, and a comparatively few deep wells. Two public supplies of a limited nature are in existence; one for Paraparaumu township and the other supplying the Waimeha Beach area of Waikanae. The former is derived from a comparatively shallow well adjacent to the Aerodrome, and is ferruginous in nature. This water is pumped into a tower and thence fed by gravity to consumers, allowing aeration of the water with resultant oxidation of the ferrous bicarbonate and precipitation of a flocculent ferric hydroxide which passes through the main to the consumer.

By contrast, the water from the Waimeha well has a neutral pH, is iron-free, and of general high quality. It is also pumped to a holding tank for gravity distribution.

The rapid expansion of the population of the area necessitates the development of new and adequate supplies of water for both domestic and firefighting purposes, and to this end the Wellington City and Suburban Water Supply Board has made a survey of likely water supplies.

Generally speaking, to derive water in this area for a public supply, three main lines of development are possible:

1. From a series of wells put down into suitable aquifers;

2. From the river, by either treatment of the water directly from the river or from intake galleries adjacent to the river;

3. From gravity schemes in the foothills of the Tararuas with either weirs or reservoirs on the tributaries of the Waikanae River. The geological maps by Oliver (1948) show that the area consists of alluvial gravels and sands overlying greywacke, with a strip of stream and river alluvium following the course of the Waikanae River to the sea. The area adjacent to the coast consists of blown sand dunes; between this and the greywacke behind is a patch of soft sandstones lying between Waikanae and Paraparaumu. Further north artesian supplies have been demonstrated, for the Foxton-Levin coastal region (Hall 1946) and in the Horowhenua district (Adkin 1948) and in the Palmerston-Wanganui basin (Ongley 1945).

TABLE 1—Data on Wells

Well	Reference (N.Z.M.S. 1 N157)	Depth (ft)	N.Z. Geological Survey No.	
PARAPARAUMU–RAUMATI AREA				
Aerodrome	528692	345	20	Near administration block
Redstone	523684	140	33	Airport manager's house
Paraparaumu (town supply)	526694	80?		Well near water tower
Paraparaumu Motor Camp A.A.	531713	150	17	
Bell	524696	110?		Area 1 Paraparaumu Township
Holmes	522698	164	21	Area 1 Paraparaumu Township
Golf Links	529707	167	22	Paraparaumu. No. 13 Tce.
Marine Gardens	520668	300?		Raumati bowling green
Raumati School	525666			Raumati
Waikanae Motor Camp A.A.	556734	120	11	Waimeha Beach
Morris	556705	64	28	Farm Otaihanga Road
Burgess	576725	—		Waikanae
County Well (Waimeha)	565730	87	26	Waikanae
Jobe (cow shed)	563716	?		North of farm Otaihanga Rd.
Jobe (shallow well)	563715	10		"
Hooper	606723	64?}		Main Road Waikanae
Heffer	608738	60?}		"
Reliance	602726	80 & 90		Reliance Factory, Waikanae
River	596713			Motor bridge, Main Highway
Pyramid	594712	shallow		Shallow well, Pyramid Cement Co.

All these areas have a similar geological history and it seemed possible that suitable aquifers would exist in the Paraparaumu-Waikanae region.

A survey was made of these wells that could be located, and 34 were chosen for examination. Shallow wells were plentiful but experience had shown that the surface waters of the area are contaminated and usually show the presence of nitrites and bacteria.

It is the purpose of this paper to describe the nature of the ground waters of this area as revealed by this investigation.

METHODS

Sampling

Bacteriological samples were taken in sterile 250 ml wide-mouthed bottles, and taps were flamed.

Chemical samples were obtained in litre or winchester bottles fitted with an inlet tube reaching to the bottom of the bottle, and an outlet tube reaching just inside the stopper. The water from the supply under examination was passed through the bottle so as to give a number of volume change-overs. This technique provided an air-free sample, preventing pH changes and precipitation of iron present in solution as ferrous bicarbonate.

Analyses

Analyses were performed according to the standard methods of the American Public Health Association "Standard Methods" (1955) with the following variations:

pH

pH was measured at the site of sampling and also at the laboratory. Some doubt may be attached to this determination, since nearly all samples came from pressure tanks, direct access to the wells not being possible. Some of the values may therefore be too high, due to possible loss of CO_2 in the pressure chambers of the pumps. Aliquots of the samples were aerated until no further change took place in pH; this was recorded as the aerated water pH (Table 2).

SULPHATE

A 100 ml sample of water was used from which the cations had been removed by ion exchange. This was evaporated to approximately 10 ml, 40 ml eythl alcohol was added, and the solution titrated with 0·0IN barium perchlorate in 80% ethyl alcohol, using thoron as the end point indicator. (Fritz and Yamamura, 1955).

DISCUSSION

Thirty-four wells were found which were of sufficient depth to warrant sampling. Twenty of these were sampled regularly and a total of 281 analyses of the waters of the region were made.

Typical analyses of these were given as follows:

Table 2 gives analyses of wells in parts per million,

Table 3 the same results in milliequivalents,

Table 4 lists a number of these analyses as ratios relative to chloride taken as unity.

The results show that the waters fall into three distinct groups, according to their provenances, which can be identified as: the Paraparaumu-Raumati area, gravels of the present Waikanae River, sands and gravels to the north of the river.

The bacteriological examination indicated the absence of coliforms, and low plate counts of less than 10 per ml at 22°C.

TABLE 2—Analyses of Well Waters

	pH	pH Aerated	pH Boiled	Fe	Alkalinity (as $CaCO_3$)	Hardness (as $CaCO_3$)	Ca	Mg (as Ions)	Na	K	Cl-	$SO_4^=$	NO_3	Specific conductance (Micromhos) at 23°C	Total solids (p.p.m)	SiO_2
PARAPARAUMU–RAUMATI AREA																
Aerodrome	6·4	7·98	9·05	2·2	168	141	40	9·6	48	6·5	36	1·0	nil	365	337	32
Redstone	7·05	8·0	8·5	2·4	168	170	44	14	91	8·9	68	2·8	nil	603	467	32
Paraparaumu (town supply)	7·0	7·95	8·5	4·0	199	156	56	3·6	53	6·5	42	2·8	nil	448	413	40
Bell	7·2	7·92	8·9	3·0	107	92	23	7·8	66	8·0	58	11	nil	333	381	40
Holmes	7·9	8·35	8·6	0·5	226	166	59	4·3	59	6·3	43	2·0	nil	462	547	40
Golf links	7·15	7·82	8·4	1·5	168	136	43	6·9	59	5·8	50	1·2	0·6	420	329	24
A.A. camp	7·1	8·02	8·72	0·66	93	132	36	7·2	63	6·0	56	1·5	nil	408	293	40
Marine Gardens	6·95	8·05	8·93	2·75	225	137	31	14	90	6·7	75	1·1	nil	555	513	40
Raumati School	7·1	—	—	2·9	36	33	6·4	4·1	57	5·4	44	5·3	nil	534	164	16
Waikanae motor camp	7·1	7·5	8·45	0·35	93	68	14	7·7	62	5·7	38	4·2	nil	263	203	20
Morris	7·1	7·63	8·32	0·70	101	83	20	7·7	56	4·8	40	11	nil	295	478	40
GRAVELS OF PRESENT WAIKANAE RIVER																
Burgess	6·55	6·7	8·6	trace	22	23	5·0	2·5	26	1·5	18	5·0	1	96	70	10
County well	7·3			0·16	111	127	50	trace	38	5·4	36	4·3	nil	315	253	16
Jobe (shed)	7·1	6·8	7·85	0·06	24	21	5·6	1·8	28	1·4	17	5·4	nil	99	96	16
Pyramid	6·7	6·75	8·85	0·60	30	29	6·2	3·2	27	1·3	20	5·0	nil	108	92	8

SANDS AND GRAVELS NORTH OF RIVER																
Hooper	6·65	7·25	7·95	trace	87	74	14	8·4	57	1·7	56	5·0	nil	277	189	32
Heffer	5·25	—	—	nil	2	28	4·6	4·1	32	3·8	25	9·0	0·15	137	107	10
Reliance (80 ft)	6·5	7·35	9·05	trace	53	77	9·2	13	53	2·1	41	11	1·8	295	203	8
Reliance (90 ft)	6·5	7·2	8·4	trace	73	85	16	11	53	2·5	49	12	0·6	295	217	20
MISCELLANEOUS																
River	7·1			0·25	23	24	5·6	2·4	23	1·3	19	7·5	nil	100	73	8
Reikorangi (rain water)					12·5	8·2	6·2	1·2	13	0·3	8	3·5				
Waikanae railway (rain water)					29	16·4	12·4	2·4	20	2·4	13	5·0				
Jobe (shallow well)	6·6	7·00	7·85	0·2	52	109	24	12	55	8·6	57	24	0·14	385	527	32

TABLE 3—Analyses of Well Waters

(Results in milliequivalents)

	Alkalinity	Hardness	Ca	Mg	Na	K	Cl^-	$SO_4^=$
PARAPARAUMU-RAUMATI AREA								
Aerodrome	3·36	2·82	2·02	0·80	2·08	0·167	1·03	0·021
Redstone	5·65	3·40	2·20	1·20	3·96	0·228	1·95	0·058
Paraparaumu (town supply)	4·00	3·12	2·82	0·30	2·30	0·167	1·20	0·058
Bell	2·14	1·83	1·18	0·65	2·88	0·205	1·66	0·238
Holmes	4·50	3·32	2·96	0·36	2·56	0·16	1·23	0·042
Golf links	3·36	2·72	2·14	0·58	2·56	0·149	1·43	0·025
Paraparaumu Motor Camp A.A.	1·86	2·64	1·84	0·80	2·74	0·153	1·60	0·031
Marine Gardens	4·50	2·74	1·54	1·20	3·92	0·172	2·14	0·023
Raumati School	0·72	0·66	0·32	0·34	2·48	0·138	1·26	0·11
Waikanae Motor Camp A.A.	1·86	1·36	0·72	0·64	2·70	0·146	1·09	0·088
Morris	2·02	1·66	1·02	0·64	2·44	0·123	1·14	0·232
GRAVELS OF PRESENT WAIKANAE RIVER								
Burgess	0·44	0·46	0·25	0·21	1·13	0·038	0·52	0·104
County well (Waimeha)	2·22	2·52	2·52		1·65	0·138	1·03	0·09
Jobe (cowshed)	0·48	0·43	0·28	0·15	1·22	0·136	0·48	0·113
Pyramid	0·60	0·58	0·31	0·27	1·17	0·034	0·57	0·104
SANDS AND GRAVEL NORTH OF RIVER								
Hooper	1·76	1·42	0·72	0·70	2·48	0·043	1·6	0·104
Heffer	0·41	0·57	0·23	0·34	1·40	0·097	0·70	0·18
Reliance (80 ft)	1·06	1·56	0·46	1·10	2·30	0·054	1·17	0·224
Reliance (90 ft)	1·46	1·67	0·79	0·88	2·30	0·064	1·40	0·246
MISCELLANEOUS								
River	0·46	0·48	0·28	0·20	1·0	0·034	0·54	0·156
Reikorangi (rainwater)	0·25	0·41	0·31	0·10	0·57	0·007	0·23	0·073
Waikanae railway (rainwater)	0·58	0·82	0·62	0·20	0·87	0·062	0·37	0·104
Jobe (shallow well)	1·04	2·18	1·2	0·98	2·40	0·22	1·63	0·50

TABLE 4—Constituents Relative to Chlorine (Milliequivalents)

Well	Alkalinity	Hardness	Ca	Mg	Na	K	SO_4
PARAPARAUMU–RAUMATI AREA							
Aerodrome	3·2	2·74	1·95	0·78	2·02	0·162	0·02
Redstone	2·9	1·74	1·13	0·62	2·02	0·117	0·03
Paraparaumu (town supply)	3·3	2·6	1·45	0·25	1·94	0·14	0·04
Bell	1·29	1·1	0·71	0·39	1·73	0·12	0·14
Holmes	3·7	2·7	2·4	0·29	2·08	0·13	0·03
Golf links	2·3	1·9	1·5	0·40	1·79	0·10	0·017
Paraparaumu Motor Camp A.A.	1·16	1·65	1·15	0·50	1·71	0·09	0·019
Marine Gardens	2·1	1·28	0·72	0·56	1·83	0·08	0·011
Raumati School	0·57	0·51	0·25	0·27	1·97	0·11	0·087
Waikanae Motor Camp A.A.	1·7	1·25	0·66	0·59	2·48	0·134	0·08
Morris	1·77	1·45	0·90	0·56	2·14	0·108	0·24
GRAVELS OF PRESENT WAIKANKAE RIVER							
Burgess	0·84	0·89	0·48	0·40	2·18	0·07	0·20
County well	2·15	2·5	2·5		1·6	0·134	0·08
Jobe (shed)	1·0	0·87	0·58	0·31	2·5	0·075	0·24
Pyramid	1·05	1·02	0·54	0·47	2·25	0·060	0·20
Hooper	1·1	0·93	0·44	0·43	1·55	0·022	0·065
Heffer	0·58	0·80	0·30	0·20	2·0	0·014	0·26
Reliance (80 ft)	0·91	1·31	0·39	0·93	1·97	0·046	0·19
Reliance (90 ft)	1·04	1·21	0·56	0·64	1·64	0·045	0·17
MISCELLANEOUS							
River	0·85	0·89	0·52	0·37	1·85	0·063	0·29
Reikorangi (rainwater)	1·08	1·78	1·34	0·43	1·5	0·03	0·31
Waikanae Railway Station (rainwater)	1·6	2·21	1·7	0·54	2·35	0·17	0·29
Jobe (shallow well)	0·64	1·34	0·74	0·60	1·47	0·135	0·31

The Paraparaumu-Raumati Area, South of the Waikanae River

All the waters of this area contain iron in the concentration of 1 to 2 parts per million in solution as ferrous bicarbonate. As they leave the ground these waters are clear in colour, somewhat acid and astringent to taste; they have all the properties of a chalybeate or ferruginous water. In contact with air the dissolved carbon dioxide is lost and the iron oxidises, a brownish flocculent ferric hydroxide being precipitated and the pH rises to values greater than 7·5. This type is characteristic of water from underground peat formations which provide quantities of carbonic acid. The existence of these latter is suggested from a study of the geological data, and the fact that peaty swamps exist at surface level in adjacent areas.

Another noticeable feature of these waters is the low amount of sulphate relative to chloride. This loss of sulphate is mostly probably due to reduction by sulphur bacteria in the presence of organic materials under anaerobic conditions, the sulphate becoming reduced to sulphide, forming insoluble ferrous sulphide and even elementary sulphur (Zobell 1946).

These reducing conditions are also reflected in the absence of nitrate in these waters. The other changes are those expected in areas of deep peats, ionic exchange processes and solubilisation of minerals by carbon dioxide. The fact that the degree of alkalinity is in most cases greater than the hardness indicates a sodium bicarbonate content. On boiling the pH rises to values of near 9 and the water attacks aluminium metal.

The Gravels of the Present Waikanae River

Water from the wells in this area – Jobe's, Pyramid, and Burgess – shows little difference in composition from the river water except for a drop in pH, due probably to the biochemical oxygen demand of the water. In passing through the ground the organic content of the water of the river is oxidised with the production of CO_2 and, because of the low alkalinity of the water, the pH drop is large.

The county well at Waimeha differs from all other well waters in the area in its ratio of calcium to magnesium. The water would appear to come through a marine bed of shells. All of the hardness of the water is attributable to dissolved calcium, there being almost a complete absence of magnesium.

Although it is on the north side of the river, and probably derived from the same strata, the water from the Waikanae motor camp resembles that from the Paraparaumu area.

The Sands and Gravels North of the Waikanae River

The water in this area is oxidised in nature as shown by the presence of nitrates and the comparatively high sulphate/chloride ratio. The chloride content of these waters suggests that they are a mixture of rain water infiltration, and water from the river through porous aquifers. The water from Heffer's well suggests a direct aquifer from the river.

The Chloride Content and the Origin of the Various Waters

It is the opinion of the authors that all these waters are of mainly rain water origin with a small contribution of connate sea water to several of the wells, particularly that at the Marine Gardens, Raumati. The rain water follows two courses, either a rapid run-off from the catchment of the Waikanae River to supply the river proper and its immediate gravel beds, or on the surrounding country and hillsides to the north and south of the river. Where there are no water courses some of the precipitated water evaporates and some soaks into the ground. This combined process will cause an increase in dissolved salt, as chloride apparently does not change through passage between the soil and rocks (Schofield 1956). If the average chloride content of the rain water is known, then, from the chloride content of the ground water, the ratio of evaporated to absorbed water may be derived. This method may possibly be a useful adjunct to the other methods of establishing the precipitation evaporation ratio.

The possibility that these higher chloride values are due to salt spray from the sea rather than evaporation is offset by the analyses of rain water from roofs in the district.

Another well on Jobe's property which is not deep enough to reach into the gravel of the river, and where the water may be expected to be completely derived from the rain water which has fallen round the area, shows the chloride to have risen to 57 p.p.m. compared with 18 p.p.m. in the river water, which also may be expected to have suffered from evaporation loss in the catchment area.

Rain water samples from the tank of the Waikanae Railway Station have shown chloride contents of 13 and 16 parts per million. These values suggest that 75% of the rain water is lost by evaporation and transpiration.

Another well in Poplar Avenue, Raumati, in an elevated situation where the water must be from local rain water infiltration, gave a chloride content of 58 parts per million.

It will be seen that with the exception of the waters near gravels of the present Waikanae River, the Marine Gardens, and Redstone's well, all contain between 36 and 59 parts per million of chloride, that is, from roughly twice to three times the chloride content of the river water. These higher values indicate that the waters are derived from rain infiltration rather than from flow from the river.

Suitability for Public Supply

The only areas worthy of consideration for public water supply are those adjacent to the river, as all waters to the south are iron containing and would need treatment both to raise the pH and remove the iron. Areas to the north of Waikanae are too far away from the areas of consumption and would need treatment to raise the pH.

All well water near the river would also need pH correction and it is considered that, owing to the nature of the country and its use, the risk of pollution from the surface is possible, and hence all well waters would need chlorination.

It may be concluded that a water development based on these underground waters will need a number of wells and require a minimum treatment of pH correction, chlorination, and possibly iron removal. For treatment of this magnitude direct orthodox filtration of the river water would be the most satisfactory method of providing a public supply.

CONCLUSIONS

Analyses of the waters of a number of wells in the Waikanae-Paraparaumu area have revealed the existence of five main classes of ground waters.

(a) The waters to the south of the Waikanae River; low pH, contain ferrous and sodium bicarbonates and low sulphate; mostly derived from rain water infiltration.

(b) The waters near the river, derived from it by percolation; low pH, low iron, oxidised, with a chloride content near that of the river.

(c) Waters from the gravel beds north of the river; low pH, no iron, sulphates high, nitrates present and chlorides intermediate in value; indicating a mixture of rain water infiltration, together with percolation through the gravels of river derived water.

(d) All surface waters from wells up to 20 ft in depth. Derived from rain water infiltration; chlorides near 57 p.p.m. Generally polluted.

(e) The Waimeha well, the water from which presumably passes through marine beds, containing shells.

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PRODUCTS OF HYDROTHERMAL HYDRATION OF CEMENTS FROM GEOTHERMAL BORES

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Summary

Samples of cement grout recovered after exposure to hydrothermal conditions in geothermal bores have been examined. Principal reaction products, apart from calcium carbonate and calcium hydroxide, were dicalcium silicate alpha-hydrate and, where a diatomite pozzolan was used, one or more of the "tobermorite group" minerals. The beta- and gamma-hydrates of dicalcium silicate and xonotlite appeared as minor constituents. The extent to which leaching of cement by geothermal fluids had occurred has also been considered.

Introduction

Cement grout has been used in steam bores at Wairakei to cement the steel casing to the country rock and also to overcome losses in circulation of drilling mud. Smith (1958) has described procedures that have been used.

The standard cement grout slurry has consisted of Portland cement with 3·19% Porangahau bentonite (3 lb per 94 lb cement) usually mixed to give a water/cement ratio of 0·64. Sometimes this ratio has been as low as 0·50. A liquid plasticising agent has been used on the basis of approximately 0·4% of the mixing water, but has been ignored in this investigation.

During the course of servicing and inspecting various bores, samples of cement grout have been recovered and these have been analysed. Samples have not been selected in a regular manner. The availability of samples of original cement grout after some time in service has been determined by the necessity for inspection work. These samples have provided the opportunity of examining grout after exposure to various hydrothermal conditions.

In addition to samples of original grout material recovered, several test samples have been examined. Two of these contained a pozzolan, while another consisted of a lime-diatomite mixture.

Samples

The source of each sample examined, in chronological order of the date taken, is as follows:

20/1–4 Bore 20 (November, 1954). Obtained adjacent to a bulge in the casing.

(1) From annulus between 10 in. and 12 in. casings, near bulge.

(2) From annulus between 8 in. and 10 in. casings, below bulge, same side.

(3) From annulus between 8 in. and 10 in. casings, below bulge, opposite side.

(4) Slush and scale adjacent to bulge.

20/A–C Bore 20 (December, 1954). Obtained from annulus between 8 in. and 10 in. casings. Three distinct adjacent layers could be observed. These were separated and analysed separately.

(A) Hard glassy layer, approx. 0·025 in. thick in immediate contact with the 8 in. casing.

(B) Intermediate, moderately hard layer, approx. 0·1 in. thick.

(C) Outer, very soft layer, approx. 0·4 in. thick.

19/A, B Bore 19 (September, 1956). Test samples of normal cement grout.

(A) Reference sample, cured in water at 18°–24°C.

(B) Moist-cured for 32 hr at 21°C, then lowered to 1,800 ft inside bore. Temperature was 240°C. Sample was recovered after two months' exposure. The bore was adjusted to permit a small bleed-off of steam during this period.

9/1–3 Bore 9 (1958). Samples exposed for about seven years. Samples represent grout recovered on excavation around well-head and were obtained from 3 ft below the cellar floor.

(1) From outside the 9½ in. O.D. surface sheathing which was completely corroded.

(2) From annulus between surface sheathing and anchor casing. The 6 in. I.D. drive anchor casing was also completely corroded.

(3) From annulus between anchor casing and the 4½ in. O.D. production casing which was lightly corroded.

50 Bore 50 (July, 1959). Sample obtained from interior of stuck drill-pipe which was recovered after about 2 years at a depth of 840 ft K.D. where the temperature was approximately 210°C.

29/B, P Bore 29 (December, 1959). Laboratory-prepared 4 in. × 4 in. cylindrical test-blocks exposed at 235°C at the bottom of the bore for six months. Before being lowered down the bore, samples were cured in water at 21°C for three days.

(B) Normal cement grout.

(P) Pozzolan cement grout consisting of 87½% Portland cement and 12½% Whirinaki micronised diatomite. (Percentages by weight.)

55/B, P Bore 55 (December, 1959). Laboratory-prepared 1 in. test cubes of standard 3% bentonite cement grout (55B) and 2 : 1 Whirinaki diatomite : hydrated lime (55P) respectively. After 2–3 days' laboratory curing

samples were lowered to 1,600 ft where they remained for 16 hr at approximately 255°C. Several sets were prepared and laboratory-cured at either 20°, 50°, or 82°C before being placed in the bore. Since no significant differences were detected due to this treatment, no distinction is made between different sets.

26 Bore 26 (January, 1960). Sample from $10\frac{3}{4}$ in.–$8\frac{5}{8}$ in. casing annulus below well-head after six years in place.

Only samples from Bores 9, 50, 29, 55, and 26 were subject to a complete examination, the earlier samples no longer being available. Hence, these grouts will be considered first.

Sample numbers were chosen to correspond with the number of the bore from which the sample was taken, to facilitate identification of sample source.

EXPERIMENTAL

Since a number of the samples contained large amounts of free water when received, it was necessary for them to be dried before representative portions were ground to pass a 100-mesh sieve. Drying was carried out in an oven at 105°C. A further sample of 29B was vacuum dried for 60 hr at room temperature over anhydrous $CaCl_2$. The free moisture contents of samples 19/A and 19/B were compared. Samples were then analysed using standard methods employed in the analysis of cements.

The Franke method (Pressler *et al.* 1956) was used for estimating free $Ca(OH)_2$. Since it is possible to remove some weakly bound lime by this method, results may be considered to be slightly high.

Insoluble residue was determined by the A.S.T.M. method C114–58, para. 28.

Differential thermal analysis (DTA) was carried out using a "Deltatherm" DTA apparatus. This has a heating rate of 9°C per minute, and with four channels and automatic operation it is able to record thermograms for four samples simultaneously. Thermograms shown in Fig. 1 each represent the mean of runs on two separate samples. Thermocouples used were mostly chrome-alumel, but in certain instances Pt–Pt/13% Rh was used. In the latter case, due to the greater mass of sample required and consequent effects on heat capacity and differences between sample blocks, the curves obtained for a particular sample with different thermocouples are not exactly comparable. Small variations in rate of paper travel have tended to expand some of the thermograms slightly.

X-ray diffraction data were obtained using a Philips X-ray diffractometer fitted with a Geiger goniometer and using $CuK\alpha$ radiation.

Refractive index measurements were performed on sample 50 when it was found to contain a high proportion of one phase.

Compressive strengths were determined on test samples 55B, 55P, 29B, and 29P. Samples 55B and 55P (1 in. cubes) were tested immediately after recovery from the bore. Samples 29B and 29P (4 in. × 4 in. cylinders) were tested at a later age after a period of conditioning in a fog curing chamber.

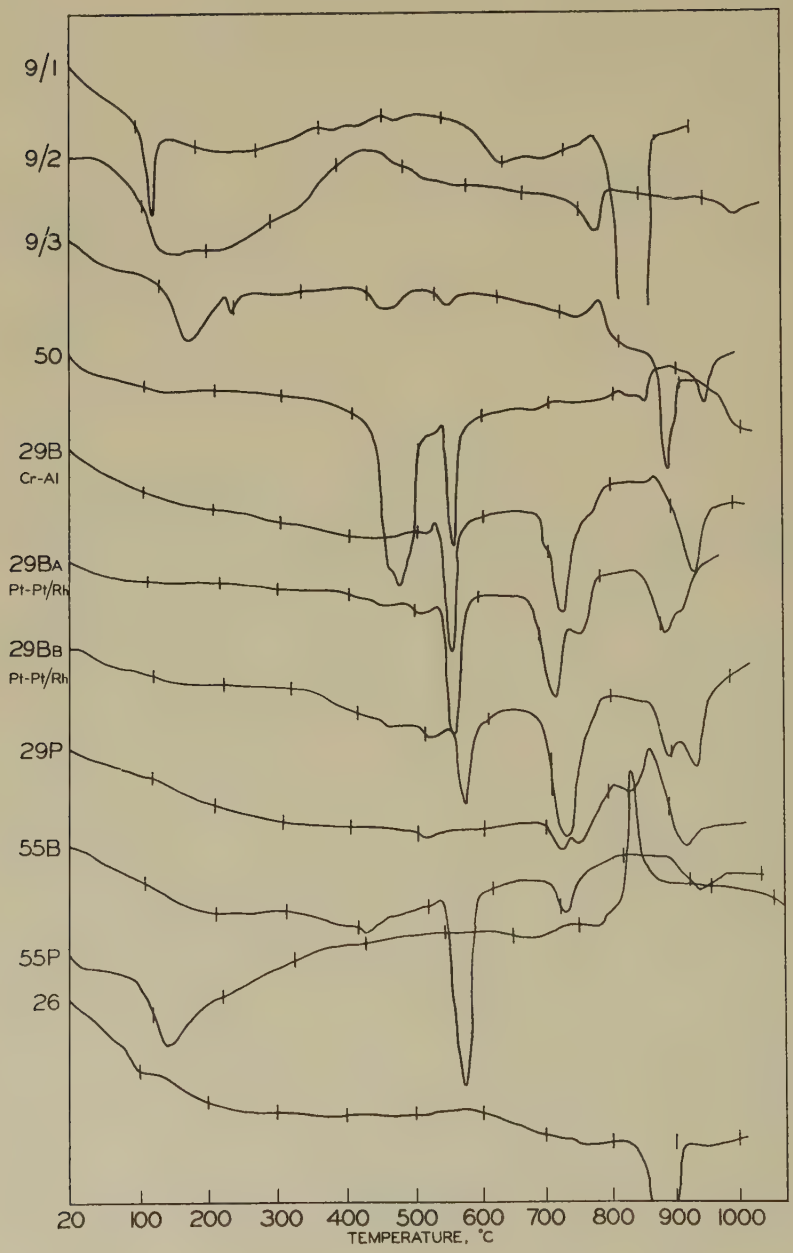

FIG. 1—DTA curves of cement grouts after exposure in geothermal bores. (29Bb – sample vacuum-dried)

RESULTS

Chemical Analyses

TABLE 1—Chemical Analyses of Grout Samples Taken 1958–60

	9/1	9/2	9/3	50	29B	29P	55B	55P	26
SiO_2	17·8	51·4	18·6	22·8	22·9	28·7	18·9	48·7	21·5
Al_2O_3	3·2	12·4	4·1	5·8	5·2	4·8	6·0	7·6	5·7
Fe_2O_3	1·8	8·9	8·1	1·8	2·1	2·3	3·3	1·8	2·3
TiO_2	0·1	0·1	0·1	0·2	0·1	0·1	0·1	0·1	0·1
CaO	44·2	14·8	28·2	54·1	56·9	52·6	56·8	23·7	46·6
MgO	1·1	1·2	0·6	1·1	1·0	0·9	1·6	0·6	1·2
SO_3	0·8	1·2	24·0	2·2	1·8	1·7	1·0	—	1·7
Alkalis*	n.d.	n.d.	n.d.	0·2	n.d.	n.d.	n.d.	n.d.	n.d.
Ignition loss	30·8	9·0	16·0	12·1	9·7	6·9	11·4	13·1	21·3
	99·8	99·0	99·7	100·3	99·7	98·0	99·1	95·6	100·4
Insol. res.	5·1	6·7	11·5	0·2	0·2	2·0	0·5	33·6	1·7
CO_2	26·2	2·9	8·3	0·6	1·6	1·3	0·7	3·9	14·9
$CaCO_3$ (from CO_2)	59·5	6·7	18·8	1·3	3·7	2·9	1·6	8·2	33·9
$Ca(OH)_2$	0·4	0·4	0·2	5·5	4·9	—	11·2	0·6	0·6

(* n.d. = not determined)

Samples 9/2 and 9/3 appear to contain substantial amounts of extraneous matter, probably drilling mud, casing corrosion products, and some of the formation surrounding the well. Excluding 9/2, 9/3, and 29P and 55P which contain pozzolan, the remaining five may be regarded as being essentially the normal 3% bentonite-cement grout, although 9/1 has an unexpectedly high insoluble residue.

Using typical analyses of both the cement and the bentonite used for grouting, the probable analyses of the original bentonite-cement grouts have been calculated and are presented in Table 2.

TABLE 2—Chemical Analyses of Bentonite-Cement Grouts Taken 1958–60 Corrected to Unhydrated Basis and Compared with Original Grout Composition

	Cement Grout*	9/1	50	29B	55B	26
SiO_2	26·3	25·3	25·4	25·0	21·0	26·8
Al_2O_3	5·1	4·6	6·5	5·7	6·7	7·1
Fe_2O_3	2·0	2·6	2·0	2·3	3·7	2·9
TiO_2	0·2	0·1	0·2	0·1	0·1	0·1
CaO	61·4	62·9	60·4	62·0	63·1	58·2
MgO	0·8	1·6	1·3	1·1	1·8	1·5
SO_3	1·5	1·1	2·5	2·0	1·1	2·1
Alkalis*	0·8	n.d.	0·2	n.d.	n.d.	n.d.
Ignition loss	1·6	1·6	1·6	1·6	1·6	1·6
	99·7	99·8	100·1	99·8	99·1	100·3

*Estimated from analyses of typical cement and bentonite used.

Likewise, the analysis of the pozzolan cement 29P has been recalculated to allow comparison with the estimated analysis of the original pozzolan-cement grout as shown in Table 3.

TABLE 3—Chemical Analysis of Pozzolan-Cement Grout Corrected to Unhydrated Basis and Compared with Original Grout Composition.

	Pozzolan-cement Grout*	29P
SiO_2	31·0	30·3
Al_2O_3	5·4	5·1
Fe_2O_3	2·0	2·4
TiO_2	0·2	0·1
CaO	56·8	55·6
MgO	0·7	1·0
SO_3	1·4	1·9
Alkalis	0·9	n.d.
Ignition loss	1·6	1·6
Total	100·0	98·0

*Estimated from analysis of typical cement and pozzolan used.

The analyses of earlier samples taken from Bores 20 and 19 during 1954–6, have also been recalculated to a 1·6% ignition loss as shown in Table 4.

TABLE 4—Chemical Analyses of Bentonite-Cement Grout Samples taken in 1954–6 and Corrected to an Unhydrated Basis.

	20/1	20/2	20/3	20/4	20/A + B	20/C	19/A	19/B
SiO_2	26·0	53·8	61·3	50·7	69·7	53·0	24·3	24·1
Al_2O_3	5·9	9·3	6·6	12·4	3·6	9·7	5·3	4·7
Fe_2O_3	3·0	2·8	2·5	3·4	1·4	2·5	1·7	2·0
TiO_2	0·3	0·4	0·2	0·4	0·1	0·4	0·2	0·3
CaO	60·6	29·2	24·4	28·7	20·5	28·2	63·8	64·1
MgO	1·1	1·1	0·7	1·3	0·4	1·1	0·4	0·4
SO_3	1·6	0·5	0·2	0·3	—	—	2·0	2·1
Alkalis*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0·7	0·6
Ignition loss	1·6	1·6	1·6	1·6	1·6	1·6	1·6	1·6
	100·1	98·7	97·5	98·8	97·3	96·5	100·0	99·9
Ignition loss*	27·5	14·0	9·2	19·1	9·2	16·8	13·6	6·6
Insol. res.*	0·7	11·5	8·6	12·3	n.d.	n.d.	n.d.	n.d.
$CaCO_3$ (from CO_2)*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	10·0	14·8
$Ca(OH)_2$*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	19·1	2·1
Moisture	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	9·8	0·7

*Determined on sample dried at 105°C.

Sample 19/B contains a very low free-moisture content by comparison with 19/A. This effect probably resulted from evaporation of water when the sample was removed from the bore.

Differential Thermal Analysis

Figure 1 reveals wide differences between the mineral compositions of various samples as indicated by their respective DTA patterns. These can be interpreted as follows:

Calcium hydroxide, which according to Webb and Heystek (1957) gives typical "characteristic" and peak temperatures of 480°C and 585°C respectively, is identified by sharp endotherms at 550°–560°C (peak temperatures) for samples 50, 29B, and 55B. In each case it may be seen that the order of concentration agrees well with that determined by chemical analysis. Although comparatively small quantities may be detected by DTA, it is possible that the $Ca(OH)_2$, which ranged between 0·2% and 0·6% with 9/1, 9/2, 9/3, 55P and 26, could represent weakly-bound lime removed during extraction.

Calcium carbonate gives typical "characteristic" and peak temperatures of 830°–920°C and 890°–1010°C (Webb and Heystek 1957). The effect of impurities in displacing the peak temperature for decomposition of $CaCO_3$ is well known. Those samples with the highest $CaCO_3$ contents among the cement grouts examined by DTA were 9/1, 9/3 and 26 with 59·5%, 18·8%, and 33·9% respectively. Thermograms of each of these samples showed large endotherms with a peak temperature of 890°C. Sample 9/2, which gave 6·7% $CaCO_3$ on chemical analysis shows a small endotherm, presumably due to calcite, at 820°C. Similarly, a smaller endotherm, at 840°C shown by sample 50 which analysed 1·3% $CaCO_3$ is also probably caused by calcite. With 3·7% $CaCO_3$ by chemical analysis, sample 29B gives a $CaCO_3$ peak having a maximum at 940°C. When a Pt–Pt/Rh thermocouple was used in place of chromel-alumel, another effect was found to be associated with the $CaCO_3$ peak. Resolution was greatest where a vacuum-dried sample was analysed. Samples 29P and 55B, with 2·9% and 1·6% $CaCO_3$ respectively determined by chemical analysis, gave relatively small $CaCO_3$ endotherms with peak temperatures 930°C and 920°C respectively. Sample 55P, with 8·2% $CaCO_3$ did not show the expected endotherm due to the presence of a strong exotherm which occurred at the same temperature.

Endotherms at approximately 100°C shown by 9/1, 9/2, 55P and 26 are due to the presence of moisture either adsorbed or remaining after incomplete oven-drying.

Sample 9/3, with 24·0% SO_3, corresponding to 40·8% $CaSO_4$, shows a double effect with peaks at 140° and 195°C, which could be due to gypsum. However, X-ray analysis could only detect anhydrite. It is presumed that, although there appears to be some discrepancy between results, this may be explained if the sample contains a mixture of gypsum, hemihydrate, and anhydrite. Since some dehydration will have occurred in removal from the bore and on oven-drying, it can be concluded that the original sample probably contained sulphate principally as hemihydrate and gypsum.

Dicalcium silicate alpha-hydrate ($C_2SH(A)$*) is characterised by an endotherm with peak temperature 470°–480°C (Kalousek and Adams 1951). Sample 50 shows a large endotherm due to $C_2SH(A)$. The irregularity on the left-hand side of the peak is typical and corresponds in shape with that obtained by Kalousek *et al.* (1954) and Midgley and Chopra (1960). Sample 9/3 shows evidence of some $C_2SH(A)$ present, while minor quantities could be present in 9/1, 29B and possibly 55B.

Dicalcium silicate beta-hydrate ($C_2SH(B)$) gives an endotherm at 540°–560°C (Kalousek *et al.* 1954) or 590°–640°C (Midgley and Chopra 1960) and is hence often difficult to identify by DTA where $Ca(OH)_2$ is also present. In view of the low value obtained for $Ca(OH)_2$ on analysis of 9/3 it is probable that it contains some $C_2SH(B)$. This compound may also be present in 29B and 29P.

Dicalcium silicate gamma-hydrate ($C_2SH(C)$) shows an endothermic peak between 730°C and 800°C (Kalousek *et al.* 1954). Sample 29B shows evidence of this compound and there is also a suggestion of its occurrence in 29P.

CSH(B) represents the monocalcium silicate hydrate which is believed to constitute the principal binder in Portland cement hydrated at normal temperatures, or at suitably low C/S ratios when hydrated at 100°–200°C. It may be either fibrous or platy, the latter being similar to the natural mineral tobermorite. Well-crystallised platy tobermorite ($C_4S_5H_5$) gives a small endotherm at 260°C but no exotherm at 800°–900°C. The associated fibrous $C_4S_5H_n$ gives a strong exotherm at 835°–865°C (Kalousek 1955, Kalousek and Roy 1957). Van Bemst (1957) found an exothermic peak over the range 835°–920°C and a second unidentified exotherm at 355°–380°C. Greenberg (1957) quotes 825°C as the temperature of the exotherm. Neese *et al.* (1957) found an endotherm at 700°C to be associated with the exotherm at 820°C. It appears that the exothermic temperature may vary between 830°C and 900°C as the C/S ratio varies between 0·8 and 1·33. Sample 55P shows a characteristically sharp endotherm due to CSH(B) over the range 750°–825°C. The peak temperature is 790°C. The small endotherm preceding the large exotherm is typical with CSH(B). An exotherm at 850°–890°C could be associated with formation of CSH(B) in 29P.

Xonotlite (C_5S_5H) was identified in sample 26 by X-ray diffraction. Mamedov and Belov (1956) found that xonotlite gave an endotherm at 770°–790°C on differential thermal analysis. Sample 26 showed a very small effect at 760°C which would confirm the above finding. Other samples, e.g., 29B and 29P also showed small endotherms possibly due to xonotlite.

X-Ray Diffraction

The chief reference used for the identification of the various hydrous calcium silicate phases from X-ray data was that by Heller and Taylor (1956). Examination of X-ray patterns obtained showed the following crystalline compounds to be present:

*The Bogue (1953) nomenclature has been used throughout. This method of formulation makes use of the abbreviations $C = CaO$, $S = SiO_2$, $H = H_2O$.

Calcite was found in all samples and appeared as the major constituent in 26.

Calcium hydroxide was detected in 50, 29B, and 55B.

Anhydrite was identified in 9/3.

$C_2SH(A)$ was found as a chief constituent of 50, and $C_2SH(B)$ and $C_2SH(C)$ appeared in minor concentrations in 29B and also probably in 29C.

CSH(B) was identified in 55P. It gave a poor, rather ill-defined pattern, but principal lines at 11·3 Å and 3·08 Å could be distinguished. Evidence for CSH(B) in 29P was somewhat fragmentary.

Xonotlite was identified in 26. It may also have been present in 50.

Quartz, present in the diatomite, appeared in 55P although there was no sign of the cristobalite originally contained in the diatomite.

Several peaks remain unidentified. These were at 5·04 Å with 26, 3·21 Å with 9/2, 3·20 Å with 55P and 2·23 Å with each of 29P and 29B, although the latter peak was stronger for 29P than for 29B. It is possible that the peak at 2·23 Å was due to Flint's CSH(A).

Refractive Index

The fine-grained texture of sample 50 made the determination of refractive index extremely difficult as it was found to be impossible to orientate any crystal. The greater portion of the sample gave a value 1·611 ± 0·003. This compares with values of $n_\alpha = 1{\cdot}614$, $n_\beta = 1{\cdot}620$ and $n_\gamma = 1{\cdot}633$ for $C_2SH(A)$ reported by Bogue (1955).

Compressive Strength

Strength measurements were necessarily confined to preformed test pieces. To a certain extent results will have been influenced by the pre-curing given the specimens. Where results are based on less than three samples, the remainder were damaged on recovery. Since it was impossible to detect any significant difference in strength between the 29B and 29P series, results obtained are presented together. Strength results and details of specimens and pre-curing are contained in Table 5.

TABLE 5—Compressive Strengths of Cement Grouts Placed in Bores 55, 29.

Sample	No. of Speci-mens	Dimensions	Pre-curing Treatment* Temp.	Time (hr)	Compressive Strength (lb/in.²) Mean	Range
55Ba	3	1 in. cubes	82°C	6½	382	380–385
55Bb	3	,,	50°C	6½	440	405–485
55Bc	2	,,	20°C	48	260	260–260
55Pa	1	,,	82°C	24	585	—
55Pb	1	,,	82°C	48	500	—
29B, P	20	4 in. × 4 in. cyls.	20°C	72	710	426–1052

*All specimens were stored at 20°C in the period (several hours) between pre-curing and lowering down the bore.

DISCUSSION

A summary of the various phases identified is given in Table 6.

Formation of one or a number of hydration products found will have been influenced by the compositions of grouts used and the conditions of exposure. The latter will include the effect of temperature, pressure, time, permeability, surface area, and location of the sample in the bore. No evidence is available to show the length of time necessary before relative equilibrium is attained, but it is notable that samples 55B and 55P, which were exposed for only 16 hours, contained similar reaction products to those recovered after several years in the bores. The products formed may be expected to be of a type similar to those formed during the autoclave curing of cement products. A knowledge of the actual identity of compounds formed during exposure in the bores is of special interest, as variations in the chemical phase composition may result in change in the physical properties of the grout. With only a limited number of samples available it cannot be definitely concluded that the results of these analyses represent the behaviour of all cement grouts used in geothermal bores. Nevertheless the findings are in general agreement with information published on the hydration of cement at elevated temperatures.

From Table 1 it may be seen that certain of the samples were contaminated with extraneous material, probably either drilling mud or country rock. Five samples (9/1, 50, 29B, 55B, and 26) of the 1958–60 series have been shown to be in a relatively uncontaminated condition and represent the products after the hydration of the standard 3% bentonite-cement grout.

From Table 6 it may be seen that calcite was present in all the samples in varied amounts. It occurred in greatest concentrations in the oldest specimens, 9/1 and 26 in which it comprised three-fifths and one-third of the samples respectively. Despite the severe carbonation of 9/1, it was found that its oxide composition was virtually unchanged, indicating that the transformation products had remained in situ. After allowing for the CaO present as $CaCO_3$, only 10·9% is available for combination with other components. Located outside the surface sheathing and fairly close to the surface, 9/1 may not at first have been subjected to hydrothermal conditions conducive to the formation of the hydrous calcium silicates normally found after curing at elevated temperatures. It is supposed, however, that with corrosion of the casing, leakage of steam about the grout may have influenced its composition. Carbonation was only very slight in other samples, but these may not have been sufficiently representative of prevailing conditions. For example, 50 was substantially protected by enclosure within the drill-pipe, 55B was exposed for an extremely brief time, and the extent to which 29B was affected in 6 months would have been influenced by the relatively low permeability of the laboratory-prepared specimens compared with that expected for grout placed under field conditions. Verbeck (1958) states that complete carbonation of Portland cement is chemically possible even at the normal CO_2 concentration found at atmospheric pressure and quotes the reaction products as $CaCO_3$, hydrous silica, alumina and ferric oxide.

TABLE 6—Cement Grouts Recovered for Examination 1958–60, Showing Products of Hydration.

SAMPLE	TYPE	CONDITIONS OF EXPOSURE			Location*	PHASES IDENTIFIED BY DTA AND X-RAY†
		Temp. (°C)	Time	Depth (ft)		
9/1	Standard cement 3% bentonite –	30–150(?)	7 yr	3	A	$\overline{CC}$, $\overline{CH}$, C_2SH(A) ?
50	,,	210	2 yr	840	D	C_2SH(A), $\overline{CH}$, $\overline{CC}$, X ?
29B	,,	235	6 mth	2180	D	$\overline{CH}$, $\overline{CC}$, C_2SH(C), (B and A) ?, X ?
55B	,,	255	16 hr	1600	D	$\overline{CH}$, $\overline{CC}$, C_2SH (C and A) ?
26	,,	180–200	6 yr	3	C	$\overline{CC}$, X
9/2	,,	100–200(?)	7 yr	3	B	$\overline{CC}$, $\overline{CH}$?
9/3	,,	180–200	7 yr	3	C	Gyp, Hemih, or Anh, $\overline{CC}$, C_2SH(A), (B) ?
29P	12½% pozzolan-cement	235	6 mth	2180	D	$\overline{CC}$, CSH(B), C_2SH(C and B) ?, X ?, CSH(A) ?
55P	2:1 pozzolan-lime	255	16 hr	1600	D	CSH(B), $\overline{CC}$, C_2SH(C and B)

*A = outside surface sheathing; B = surface sheathing/anchor casing annulus; C = anchor casing/production casing annulus; D = inside bore.

†Phases shown in order of apparent decreasing concentration. In addition to the Bogue (1953) nomenclature, the following abbreviations are used:

$\overline{CC}$ = $CaCO_3$, $\overline{CH}$ = $Ca(OH)_2$, X = Xonotlite, Anh = $CaSO_4$, Hemih = $CaSO_4.\frac{1}{2}H_2O$, Gyp = Gypsum.

It is assumed that the high concentration of calcium sulphate in 9/3 resulted from the action of H_2S which leaked through the corroded production casing and was subsequently oxidised.

Calcium hydroxide was found in several of the samples. It would have been formed as a product of hydrolysis of the C_3S and C_2S, mineral constituents of Portland cement. Its presence would favour the formation of the high-lime calcium silicate hydrates.

Except in the case of the two oldest samples, 9/1 and 26, wherever Portland cement (3% bentonite) grouts were used, dicalcium silicate hydrates were shown to be present. The most abundant of these appears to have been the alpha-hydrate, $C_2SH(A)$ which comprised at least two-thirds of sample 50. DTA suggests that there may be some $C_2SH(A)$ in 9/1; however it has no doubt suffered decomposition by CO_2 with the formation of $CaCO_3$ and probably hydrous silica. Although it also contained a large proportion of $CaCO_3$, 26 contained xonotlite in preference to the dicalcium silicate hydrates; this may be explained by its prolonged exposure to a higher temperature than was the case with 9/1. Each of the compounds $C_2SH(A)$ and C_5S_5H are found in steam-cured cement products, the concentrations depending upon the temperature and pressure used. $C_2SH(A)$ has been identified as a low-strength cementing material (Taylor 1952: Saunders and Smothers 1957) while xonotlite, which has been described as one of the most easily synthesised of the hydrous calcium silicates (Kalousek 1952), is characterised by its physical stability at temperatures below 710°C. Strength measurements did not extend to those samples in which significant quantities of these minerals were present.

Where they were also identified, the beta- and gamma-hydrates of dicalcium silicate, $C_2SH(B)$ and $C_2SH(C)$ respectively, appeared as very minor constituents. Kalousek (1952) states that the stable phase formed on autoclaving high-lime mixtures in the lime-silica-water system is $C_2SH(A)$. He also considers that it forms in the absence of an excess of $Ca(OH)_2$ and that $C_2SH(B)$ and $C_2SH(C)$ are simply transition products during its formation. Heller and Taylor (1952) always obtained $C_2SH(A)$ on hydrolysis of C_3S between 100° and 200°C and assumed that it is stabilised in the presence of $Ca(OH)_2$. It is notable that wherever $C_2SH(A)$ was found in the present samples, $Ca(OH)_2$ was also present.

The formation of $C_2SH(A)$ has been stated to confer increased resistance to attack by aqueous sulphate solutions. (Thorvaldson and Shelton 1929; Vigfusson *et al.* 1934). It may thus be assumed that, if $C_2SH(A)$ had formed in sufficient concentrations, corrosion due to H_2S and sulphate would have been retarded.

Where $C_2SH(B)$ and $C_2SH(C)$ were detected (sample 55P), no appreciable $Ca(OH)_2$ was found. Also, while studying the lime-silica-water system, in this case at 100°–220°C, Assarsson (1958) concluded that the first stage of hydration was the formation of an unstable "phase B" which under favourable conditions and with a sufficiently high CaO/SiO_2 ratio formed $C_2SH(A)$ or $CSH(B)$. The third stage was the transformation to $C_2SH(B)$ and possibly C_5S_5H. Results obtained for the grout samples

examined showed that $C_2SH(C)$ occurred in greater concentrations than $C_2SH(B)$.

The formation of the monocalcium silicate CSH(B) in 55P and 29P is in accordance with the low CaO/SiO_2 ratios, although with 29P this was as high as 1·8. However, where Portland cement is the source of lime, allowance must be made for lime which will have reacted with aluminium, iron, and sulphate in the cement. Differences between the shapes of the exothermic peaks obtained on DTA of 29P and 55P may be seen in Fig. 1. Sample 29P, having the higher CaO/SiO_2 ratio, tends towards the rounded exotherm obtained by Kalousek and Prebus (1958) at 880°–910°C for a CaO/SiO_2 ratio of 1·5 in a lime-silica-water mixture. These authors report a prolonged exothermic effect at 870°–920°C which they claim to be due to an alumina-bearing hydrous calcium silicate. They also distinguish between three very similar hydrous Ca silicates present in set cement, viz. two minerals of the CSH(B) or "tobermorite group" and C_2SH_2, with basal spacings of 14 Å, 11 Å and 10 Å respectively. The name "tobermorite" is correctly applied only to the 11 Å compound although the group includes 14 Å, 12·5 Å, 11 Å, 10 Å and 9 Å hydrates. Kalousek and Prebus present DTA data indicating that tobermorite is characterised by an endotherm at 260°C and the 0·8–1·33 hydrate by an exotherm between 835° and 900°C. Midgley and Chopra (1960), on the other hand, showed an exotherm at 825°C for tobermorite and a similar exotherm for CSH(A). X-ray data for 55P show lines at 11·3 Å and 3·08 Å suggesting that tobermorite may have formed although no thermal effect was obtained at 260°C. This inconsistency could be partially resolved if the sample contained proportions of each of the 11 Å and 14 Å hydrates. Neese *et al.* (1957) emphasise the limitations of X-ray methods of identifying calcium silicate hydrates and base conclusions of their investigations on DTA, optical properties and chemical analysis.

It is interesting to note that the strength of sample 55P was greater than that of 55B. Sample 55P, with CSH(B) as the main binder contained no appreciable $Ca(OH)_2$. Its fairly substantial $CaCO_3$ content would probably be due to carbonation of lime during preparation of specimens. Sample 55B most likely contained $C_2SH(A)$ as a principal cementing agent. It also had a large amount of uncombined $Ca(OH)_2$.

Table 6 does not list any alumina- or iron-bearing phases. The R_2O_3 constituent in the original Portland cement will have been approximately 7·5%. Presumably the products formed were present in too small concentrations to permit their identification. Nevertheless, if a significant amount of C_3AH_6 (the product of hydrothermal hydration of C_3A) remained, it should have been detected by DTA, as Kalousek *et al.* (1949), and Kalousek (1952) found that 0·5–1·0% quantities could be identified by means of the endotherm at 280°–310°C. Thus it is probable that these constituents do not form crystalline products under these conditions. However, Kalousek (1957) and (Neese *et al.* 1957) have stated that small quantities of Al_2O_3 and Fe_2O_3 may be contained in the CSH(B) crystal lattice without a noticeable change in the properties of the products.

Considerable interest is attached to the degree to which leaching of the

cement grout may be occurring due to solution in geothermal fluids. The samples examined will have varied considerably in permeability, and time and conditions of exposure. The older samples 9/1 and 26 nevertheless give an indication of the extent of this action.

It may be expected that, where solution has taken place, there should be a reduction in the CaO content or an increase of the $(SiO_2 + R_2O_3)/CaO$ ratio of the sample.

TABLE 7—Oxide Ratios Based on Data on 1958–60 Samples in Table 2

	Cement Grout	9/1	50	29B	55B	26
$\frac{SiO_2 + R_2O_3}{CaO}$	0·564	0·518	0·565	0·534	0·499	0·634

Table 7 shows that leaching of lime has been most severe with 26. On the other hand, 9/1 does not show a similar effect. Ratios must be regarded as approximate, as 55B, exposed for only 16 hr, had a ratio considerably lower than estimated for the original grout.

Similar ratios calculated for the 1954–6 samples from Bores 19 and 20 show that, after 2 months at 240°C 19/B has a ratio of 0·485, or substantially the same as the laboratory-cured 19/A of 0·494. Sample 20/1 at 0·581 probably shows signs of minor leaching of lime.

The interpretation of analytical data for these latter samples is complicated due to (a) uncertainty as to whether the high insoluble residues for 20/2–4 result from leaching of the cement or from the presence of drilling mud, and (b) the absence of values for the insoluble residues of other grout samples in the same series. (Samples were not available for reinvestigation.) Where leaching has occurred and lime alone removed, the SiO_2 and R_2O_3 should remain in relatively constant proportions.

TABLE 8—Oxide Ratios Based on Data on 1954–6 Samples in Table 4

	20/1	20/2	20/3	20/4	20/A + B	20/C	19/A	19/B
$\frac{SiO_2 + R_2O_3}{CaO}$	0·581	—	—	—	—	—	0·494	0·485
$\frac{SiO_2}{R_2O_3}$	2·82	4·31	6·59	3·13	13·7	4·21	3·38	3·44
$\frac{Al_2O_3}{Fe_2O_3}$	1·97	3·32	2·64	3·65	2·57	3·88	3·12	2·35

The very high SiO_2/R_2O_3 ratio shown for 20/A + B in Table 8 was apparently caused by the deposition of silica (as layer A) at the interface

between casing and grout. It may be concluded from the range of Al_2O_3/R_2O_3 ratios that the grout samples were not appreciably contaminated with corrosion products of the casing.

The pozzolan cement grout 29B showed no real change in oxide composition after 6 months' exposure.

The effect of increasing temperature in the bore on equilibria conditions should be to lower the pH slightly, but not sufficiently to cause solution of hydrated cement. It is not considered that any of the salts dissolved in Wairakei bore waters, of which the chief is NaCl 0·3% (Smith 1958) should affect the condition of the cement grout deleteriously.

CONCLUSIONS

The principal products of hydration of cement samples exposed under various hydrothermal conditions, apart from calcium carbonate and hydroxide, were the low-strength dicalcium silicate alpha-hydrate which comprised about two-thirds of one cement grout and, where diatomite pozzolan was used, one or more of the "tobermorite group" of minerals, according to the CaO/SiO_2 ratio. Xonotlite and the beta- and gamma-hydrates of dicalcium silicate were detected in lesser amounts.

Carbonation of cement grout was most severe in samples recovered from near the surface and which were in contact with ground water. Where the inner casing of one bore had corroded away, the grout was composed of over 40% calcium sulphate due to leakage of H_2S from the bore.

No evidence was found to indicate the form in which aluminium or iron compounds were present.

Any leaching of cement by geothermal fluids was of minor consequence. It may possibly be chiefly confined to outer surfaces. A sample of pozzolan-cement grout showed no sign of leaching after exposure inside a steam bore at 235°C for 6 months.

It is thought that, where a layer of silica occurs on the grout, this could be derived by deposition from the geothermal fluid rather than by leaching of other constituents from the grout.

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TOXICITY OF BORIC ACID AND ZINC CHLORIDE TO LARVAE OF THE TWO-TOOTH LONGHORN, *AMBEODONTUS TRISTIS* F.

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Summary

The toxicity of boric acid and zinc chloride to larvae of the two-toothed longhorn beetle, *Ambeodontus tristis* F. has been determined. Larvae did not survive in blocks of sapwood kahikatea (*Podocarpus dacrydioides*, A. Rich.) containing 0·142% or more boric acid or 0·41% zinc chloride. The method of uniformity impregnating blocks and the testing technique described are suitable for determining toxicity of other water soluble preservatives to this insect.

Introduction

The only published information on the toxicities of timber preservatives to larvae of the two-tooth longhorn, *Ambeodontus tristis* F., is a single determination of the toxicity of boric acid, obtained during exploratory work on methods suited to testing toxicity of wood preservatives with this insect (Spiller, 1952). The present paper gives the results of a larger experiment designed to redetermine the toxicity of boric acid and to determine the toxicity of zinc chloride. The testing technique described in the earlier note was modified and can now be considered satisfactory for routine testing. The results obtained show that larvae of this insect are rather tolerant to zinc chloride and that boric acid is less toxic to the larvae than indicated earlier. The effectiveness of the standard timber preservation treatment is not impaired by this redetermination.

Methods and Materials

The boric acid and zinc chloride employed were A.R. quality. The test timber was sapwood kahikatea (*Podocarpus dacrydioides*, A. Rich.). Three boards, designated X, Y, and Z, from different sources were used to prepare test blocks. Blocks from the different boards were identified by the board letters and kept separate throughout the experiment. Test blocks, which had oven dry weights of about 100 g, measured 14·5 cm × 5 cm × 4 cm, the the shortest distance being along the grain.

Blocks were treated and handled as follows: Six randomly selected blocks from one board were numbered, oven dried, weighed and placed in a large beaker with lead counterweights. The counterweights prevented the blocks floating later. The beaker was placed in a large vertical vacuum-pressure cylinder, which was sealed and then evacuated at 28 in. for 15 minutes. Then the required treating solution was run into the beaker under full

vacuum, the vacuum released and the air pressure raised to 150 lb per sq. in. for 30 min. Blocks remained in the treating fluid for 30 min after the pressure was released. They were then removed from the treating fluid, drained on a towel for 5 min, and weighed. Finally, blocks were stored in the laboratory for two days and then dried and stored in a dry-air drying room.

Loadings (that is, the percentage weight/weight of preservative on oven dry wood) of individual blocks were computed in the usual manner from the known oven dry weight, weight of solution absorbed, and the weight concentration of treating solution.

A few days before blocks were infested they were removed from the drying room and placed in a testing room maintained at 24°C and about 95% relative humidity. At that time, egg transfer sites $\frac{1}{32}$ in. wide and $\frac{1}{16}$ in. deep and long were made in one of the end grain surfaces.

Each day eggs were collected from mated *Ambeodontus tristis* females individually caged in petri dishes with small blocks of wet wood. After these eggs had been pooled and randomised, twelve were evenly distributed on a block, one egg to a transfer site. Then eggs were placed on the next block, and so on, day by day, until all were infested. As each was infested, blocks were returned to the testing room where they remained for three years. They were then stored in an insectary, recording finally taking place five years after the experiment was set up. During this period all larvae had either transformed to adults and emerged (mainly after two years) or had succumbed to the effects of the preservative.

Two criteria of infestation have been employed, (1) the presence of exit holes in the block surface, and (2) when no emergence occurred, the presence of substantial tunnels within the block indicating that one or more larvae had established and survived for a considerable time.

RESULTS

Detailed recordings of blocks are given in Table 1 for boric acid and Table 2 for zinc chloride. Fig. 1 shows sections of infested blocks and is referred to in the discussion.

DISCUSSION

Larvae established well in untreated blocks and in blocks with low loadings of preservative. Some blocks were so extensively tunnelled (Fig. 1, A, B) that portions crumbled and fell away. There can therefore be no doubts about the suitability of egg transfer as a method of infesting the blocks, or of susceptibility of the test blocks to larval attack, or of the adequacy of the environmental conditions for growth and survival of larvae. The technique presented here can in fact be considered entirely suitable and not too laborious for the routine determination of the toxicity of water soluble preservative to this insect.

TABLE 1—Toxicity of Boric Acid to Larvae of *Ambeodontus tristis*

Average* Loading	Board	Condition of Block†					
0·010 ..	Y	E	E	E	E	E	E
0·011 ..	Z	E	E	E	E	E	E
0·012 ..	X	E	E	E	E	E	E
0·021 ..	Y	E	E	E	E	E	E
0·021 ..	Z	E	E	E	E	E	E
0·024 ..	X	E	E	E	E	E	E
0·041 ..	Z	E	E	E	E	E	E
0·042 ..	Y	E	E	E	E	E	E
0·046 ..	X	E	E	E	E	E	E
0·082 ..	Z	E	E	I	I	I	O
0·083 ..	Y	E	E	E	E	E	O
0·091 ..	X	E	I	I	O	O	O
0·124 ..	Z	I	O	O	O	O	O
0·129 ..	Y	E	O	O	O	O	O
0·142 ..	X	O	O	O	O	O	O
0·16 ..	Z	O	O	O	O	O	O
0·17 ..	Y	O	O	O	O	O	O
0·18 ..	X	O	O	O	O	O	O
0·21 ..	Z	O	O	O	O	O	O
0·21 ..	Y	O	O	O	O	O	O
0·23 ..	X	O	O	O	O	O	O
Untreated ..	X	E	E	E	E	E	E
„ ..	Y	E	E	E	E	E	E
„ ..	Z	E	E	E	E	E	E

*Average percentage of boric acid W/W on oven dry wood, for the six uniformly treated blocks.

†Six blocks for each average loading.

E = With one or more exit holes.

I = With substantial tunnels but no exit holes.

O = Undamaged.

The treating schedule adopted fills the whole of the void volume of the block with treating solution (cf. Harrow, 1951) and should result in complete and uniform penetration of the block with preservative. No portions of blocks were chemically analysed to provide a formal proof of this assumption but very probably such uniformity was achieved, for although longitudinal sections of infested treated blocks sometimes revealed a characteristic pattern of larval workings (Fig. 1, C, D, E. F) suggestive of an avoidance reaction in a concentration gradient of preservative, a similar pattern of tunnels occurred in lightly infested untreated blocks (Fig. 1, G, H, I). In heavily infested blocks, either treated or untreated, any pattern is partially obscured by the maze of tunnels (Fig. 1, J, K) some of which extend to within one or two millimetres of the surface (Fig. 1, J. K, L, M).

Some may consider it largely a matter of choice whether the criterion of effectiveness is that of prevention of emergence or prevention of infestation, but in blocks from which no emergence occurred occasional larvae became well grown and made large and extensive tunnels before they died (Fig. 1, E, N, O). If such tunnels were found during sawing or working preserva-

TABLE 2—Toxicity of Zinc Chloride to Larvae of *Amboedontus tristis*

Average* Loading	Board	Condition of Block†					
0·052 ..	Y	E	E	E	E	E	E
0·052 ..	Z	E	E	E	E	E	E
0·059 ..	X	E	E	E	E	E	E
0·10 ..	Y	E	E	E	E	E	E
0·10 ..	Z	E	E	E	E	E	E
0·12 ..	X	E	E	E	E	E	E
0·15 ..	Z	E	E	E	E	E	E
0·16 ..	Y	E	E	E	E	E	E
0·18 ..	X	E	E	E	E	E	E
0·20 ..	Y	E	E	E	E	E	I
0·20 ..	Z	E	E	E	E	E	I
0·24 ..	X	E	E	I	O	O	O
0·26 ..	Z	E	I	I	O	O	O
0·26 ..	Y	E	E	E	I	O	O
0·29 ..	X	O	O	O	O	O	O
0·31 ..	Y	E	E	I	I	O	O
0·32 ..	Z	E	I	I	I	O	O
0·35 ..	Z	I	I	I	O	O	O
0·36 ..	X	I	O	O	O	O	O
0·36 ..	Y	I	O	O	O	O	O
0·41 ..	X	O	O	O	O	O	O
Untreated ..	X	E	E	E	E	E	E
„ ..	Y	E	E	E	E	E	E
„ ..	Z	E	E	E	E	E	E

*Mean percentage of zinc chlorides W/W on oven dry wood, for the six uniformly treated blocks.

†Six blocks for each average loading.

E = With one or more exit holes.

I = With substantial tunnels but no exit holes.

O = Undamaged.

tised timber it would be natural to conclude that the timber had not been treated or that treatment was faulty. For this reason it seems necessary to insist that the minimum toxic loading be taken as that at which no substantial tunnels are formed. Even in blocks with very heavy loadings tunnels of about a millimetre diameter were sometimes found as far as 15 to 20 mm from the infested surface. These tunnels usually ran straight in from the infestation site, a small dead larva being found in the blind end. Obviously these small tunnels were made by the newly hatched larvae and do not indicate that the loading is inadequate to prevent infestation.

Table 1 shows that loadings of boric acid of 0·142% and higher gave a complete kill, while loadings of 0·129% and below permitted some larvae to survive or emerge. This minimum toxic loading of between 0·14% and 0.13% boric acid is considerably greater than the earlier determination of the minimum toxic loading as between 0·092% and 0·046% boric acid, with probable value of 0·066% (Spiller, 1952). However, such upward revision of values is not unexpected considering the larger number of boards, blocks, and larvae used in the present experiment. It might well be accounted

FIG. 1—Larval workings of *Ambeodontus tristis* in treated and untreated blocks.

A, B: Across grain sections through mid line of block.

L, M: Across grain sections, 5 mm from block face.

Remainder: With grain sections through mid-line of block. In blocks other than A, L, M the tightly packed frass or partially digested wood has been dug out of the larval workings.

for by the low innate susceptibility to attack of the original test blocks, for, although nothing is known of how toxicity is affected by susceptibility of blocks to attack, it will not be surprising if the minimum toxic loading is lower in blocks that are not very susceptible.

Table 2 shows that zinc chloride is not very toxic to *Ambeodontus* larvae, emergence or prolonged survival occurring with all blocks containing 0·20% zinc chloride or less, and in some blocks at each loading up to 0·36% zinc chloride. The only loading at which all blocks were undamaged was 0·41% zinc chloride. Thus, on the results of this trial, the minimum toxic loading is between 0·41% and 0·36% zinc chloride.

It is of interest to compare the relative toxicity of boric acid and zinc chloride to larvae of the three wood destroying beetles so far used as test insects. As percentage of boric acid or zinc chloride on oven dry wood, the minimum toxic loadings are: Boric acid, *Lyctus brunneus* Steph., between 0·16% and 0·13% (Cummins, 1939); *Anobium punctatum* De Geer, between 0·043% and 0·022% (Spiller, 1948); and *Ambeodontus tristis* F., between 0·14% and 0·13% (Spiller, this paper); Zinc chloride, *Lyctus brunneus,* between 1·22% and 0·85% (Cummins, 1939); *Anobium punctatum,* between 0·18% and 0·11% (Spiller, 1950) and *Ambeodontus tristis,* between 0·41% and 0·36% (Spiller, this paper). Thus both materials are most toxic to larvae of *Anobium,* boric acid is about equally toxic to *Lyctus* and *Ambeodontus,* and toxicity of zinc chloride to *Ambeodontus* is intermediate between its toxicity to *Lyctus* and *Anobium.*

Although zinc chloride was included in the New Zealand Code of Practice for Timber Preservation (Anon., 1953a) as one of the available water soluble preservatives, the material has not been used by the New Zealand timber preservation industry and at the time of writing there seems little likelihood that it will be introduced. Therefore the toxicity figures obtained in this study are not of immediate practical significance.

On the other hand, the boric acid toxicity figures obtained in this study are of considerable practical significance, for boric acid (and other boron compounds) has been and is extensively used for timber preservation in New Zealand, the current annual output of boron preservatised timbers being approximately 100 million board feet. Some of this timber is pressure treated, but much is green timber treated by some process involving diffusion during storage, before air or kiln drying. Whatever process or variant is adopted, a minimum requirement is that the core of each board contain not less than 0·2% boric acid by weight (Anon., 1953b; Harrow, 1954). The results in Table 1 show that 0·2% is in excess of that concentration at which *Ambeodontus tristis* larvae are able to survive. It follows therefore that timber treated according to the present New Zealand specifications and requirements for preservation of timber with boric acid, will be adequately protected against attack by *Ambeodontus tristis* larvae.

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FIRE HAZARDS IN THE USE OF FERTILISERS CONTAINING ELEMENTAL SULPHUR

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Summary

Fertilisers containing elemental sulphur are being used to an increasing extent in New Zealand for aerial-topdressing. Commercial mixtures were tested for explosion, inflammability, and electrostatic discharge risks, and it was shown that serious explosion danger is involved in the use of fertilisers containing a high proportion of sulphur. The limiting safe sulphur content is inversely proportional to the specific surface of the sulphur. Superphosphate and gypsum were found to be more effective as explosion dampers than limestone. The maximum safe concentration is considered to be 23% commercial screened sulphur. It is suggested that gypsum should be used as diluent in highly sulphur-deficient areas, and superphosphate as diluent in all other areas.

INTRODUCTION

Plants contain approximately twice as much sulphur as phosphorus, and Bogdanov (1899) first pointed out the possibility of sulphur-deficiency in grasslands. In New Zealand, Doak (1929) demonstrated a response of lucerne to sulphur addition, but it was only when Lobb (1953) publicised the beneficial effect of sulphur on clover, that New Zealand farmers became concerned. Since then the Department of Agriculture has encouraged the use of sulphur-containing fertilisers and laid down sulphur-response experimental plots throughout the country, while Walker and Adams (1958) have done a considerable amount of work on the recovery of various forms of applied sulphur and their effect on clover growth.

In 1959 approximately 1,000 tons of elemental sulphur was employed as a fertiliser in New Zealand, most of it in the form of mixtures with superphosphate containing 18% or less of sulphur, although smaller quantities of special mixtures containing up to 36% sulphur were also used. In areas that apparently needed no phosphate, mixtures containing up to 36% sulphur in limestone (a less expensive diluent) were employed. Most of this fertiliser is applied by aerial topdressing.

Elemental sulphur is inflammable, and while fires in sulphur stored in bulk will only rarely cause serious damage, numerous accidents with explosions in sulphur dust-clouds have been reported. It is possible that such dangerous clouds might occur below an aircraft dropping fertiliser, where a hot exhaust pipe or sparks resulting from a stone striking the hopper could cause an explosion. The present work was undertaken to investigate what fire hazards are associated with sulphur-containing fer-

tilisers that are being dropped from low-flying aircraft. The use of gypsum, instead of limestone or superphosphate, is considered as an alternative diluent for elementary sulphur.

Australian Fertilisers Ltd. (1958) have investigated the explosion hazard attached to the aerial spreading of their sulphur-fortified superphosphate, which contains 18% sulphur and 82% superphosphate. They tested various mixtures in a Godbert apparatus (1934) for explosion risks, obtained sieve analyses of a number of commercial sulphur and superphosphate samples, and found that — 100 mesh sulphur was the main danger, while —100 mesh superphosphate constituted the main protective agent. Their sulphur-fortified superphosphate is therefore made from sulphur with a sieve-analysis of specified maximum fineness and superphosphate with a specified minimum fineness, each having one chance in a hundred of occurring by random selection from stocks. As added safeguards they recommended (1) that only dark sulphur be used in mixtures for aerial spreading, and (2) that quality control be adopted to see that — 100 mesh fraction of sulphur is kept below 15% and that the proportion of sulphur in the mixture as bagged is not greater than 23%.

In the present work, explosion tests were done on commercial sulphur-containing fertilisers and synthetic samples with various sulphur sieve fractions, in each case using a range of diluents. Some work on inflammability and electrostatic discharge hazards was also performed and suggestions suitable for New Zealand conditions are made.

EXPERIMENTAL PROCEDURE AND RESULTS

Five-pound samples of typical sulphur-containing mixtures and screened sulphur were supplied by different fertiliser companies for estimation of ignition hazards. The diluents employed, additional samples of superphosphate, limestone, and various grades of gypsum were also obtained. Nine diluents, 18 commercial mixtures and 4 sulphurs (3 bright and 1 dark) were received.

Table 1 shows sieve analyses with BS sieves, and moisture contents (obtained by prolonged drying at 60°C) of the diluents and a range of commercial fertiliser mixtures. Sulphur sieve analyses are shown and a "fineness factor" F as defined by Mason and Wheeler (1936a) is also included (see Discussion). Details of the composition (as stated by the supplier) of all mixtures received are included in Table 5.

Explosion Tests

The mixtures received were tested in a standard Godbert inflammability apparatus (1952) which was designed to give results equivalent to tests on coal dust in a 4 ft diameter experimental explosion gallery. A measure of the explosion hazard in dust clouds can thus be obtained.

TABLE 1—Moisture Content and Sieve Analysis of a Range of Diluents, Fertiliser Mixtures, and Sulphurs

Sample No.	Description	% Moisture Content (Dried at 60°C)	% Sieve Analysis on Dried Material (B.S. Sieve Sizes)					Fineness Factor F
			+22	−22 +60	−60 +120	−120 + 240	−240	
1	Superphosphate, fertiliser company	5·8	44·7	31·1	13·3	5·2	5·7	0·338
2	Superphosphate, seed merchant	3·5	0·5	49·6	27·3	15·7	6·9	0·538
3	Limestone, fertiliser company	3·2	25·1	46·6	15·8	7·4	5·1	0·378
4	Limestone, fertiliser company	13·3	13·0	41·7	33·0	10·0	2·3	0·411
5	Limestone, seed merchant	0·4	0·1	14·8	61·3	20·4	3·5	0·613
6	Limestone, finely ground	0·2	0·0	0·0	20·0	20·0	60·0	1·500
7	Gypsum, coarse commercial, 17·6% S	0·5	47·6	30·1	15·2	4·4	2·7	0·279
8	Gypsum, commercial, 17·6% S	0·3	10·9	60·2	10·2	5·1	13·6	0·512
9	Gypsum, finely ground, 17·6% S	0·2	0·0	24·8	29·6	24·4	21·2	0·869
13	800 lb S/ton in superphosphate	1·5	37·4	38·8	12·3	5·9	5·6	
15	400 lb S/ton in superphosphate	2·0	18·4	37·6	18·2	10·0	15·8	
17	400 lb S/ton in cobalt-superphosphate	2·2	55·6	29·8	6·7	3·6	4·3	
23	600 lb S/ton in limestone	0·5	12·0	44·0	20·2	13·0	10·8	
25	800 lb S/ton in limestone	0·5	25·5	51·0	14·9	5·4	3·2	
26	600 lb S/ton in limestone	2·7	12·0	23·8	30·8	22·9	10·5	
27	600 lb S/ton in limestone	11·5	15·9	46·2	30·0	6·5	1·4	
A	Sulphur, bright	0·0	1·2	61·4	19·6	10·4	7·4	0·482
B	Sulphur, dark	0·0	28·6	40·1	16·0	9·0	6·3	0·407
C	Sulphur, bright	0·0	10·7	58·3	20·1	8·7	2·2	0·366
D	Sulphur, bright	0·0	18·7	55·1	16·0	5·9	4·3	0·359

In this apparatus a fixed volume of air, held at a pressure of 18 in. mercury above atmospheric, is released and carries a 1 g sample into a tube furnace, which has a temperature in the vicinity of 820°C. The temperature was adjusted as required, after testing the apparatus with a standardised coal-dust mixture, which is known just to explode in the experimental gallery. The test relies on visual judgment of presence or absence of a flame. In a just non-explosive sample, where no flame is observed in a large number of tests, an increase of one-fifth of the amount of sulphur present is usually sufficient to give a definite explosion in every test. An experienced operator can reproduce limiting sulphur contents (in which explosions occur in half the tests) to about $\pm 3\%$ sulphur in mixtures containing 40% sulphur, $\pm 2\%$ in mixtures with 20% sulphur and $\pm 1\%$ below 10% sulphur. The limiting sulphur content can, therefore, be determined to about one-tenth of the amount of sulphur present.

Results were also obtained in a smaller modification of the apparatus (1934), which was designed to give results equivalent to the standard apparatus. The standard apparatus can only be used with fractions sieved to — 22 mesh, but in the small apparatus the sample, as received, and also each sample sieved to — 22 mesh could be tested.

For every group of fertiliser samples as received from one source, mixtures were diluted, using superphosphate 1 and 2 (see Table 1) and limestone 3 and 5 to find the limiting concentrations of sulphur that gave flames in more than half the tests. A number of tests were performed with these limiting concentrations, but no attempt was made to evaluate the efficiency of mixing in the commercial samples, by statistical analysis of large numbers of results. Samples as received and dilutions of all high sulphur samples in the same group gave very consistent results for limiting concentrations of sulphur. These are summarised in Table 2 and are shown as lb sulphur/ton mixture. Sample 27, which had a particularly high moisture content (11·5%), was also tested after drying at 60°C, and diluted with dried limestone 4. (Except where stated, all mixtures were undried.)

The effect of particle size of sulphur on explosion hazards was next investigated with synthetic mixtures. Two varieties of sulphur, one bright and one dark, were sieved into five fractions, and each fraction was tested with a number of diluents (sieved to — 22 mesh). In addition, the sieve fractions of the dark sulphur sample were remixed to give samples for testing of "specified" sulphur, as defined by Australian Fertilisers Ltd. (1958), and also of what this company considered "average" sulphur. The original Australian Fertiliser (1958) Tyler sieve analyses were converted to equivalent BS sieve sizes, and are given in Table 3. Table 4 summarises results obtained, which are expressed as per cent by weight of sulphur in the total mixture. These results were all obtained on the small Godbert apparatus (1934). A number of tests performed on the standard Godbert apparatus (1952) gave similar results.

TABLE 2—Limiting Sulphur Concentrations (lb/ton mixture) that Pass Half the Godbert Tests in Each Group of Samples Received

Sample Numbers in Group		Standard Apparatus −22 Mesh	Small Apparatus				
			As Received			−22 Mesh	
	Diluent Superphosphate	2	1		2	1	2
10–13	Sulphurised superphosphate	550	550		650	600	600
14–16	,, ...	>400	—		>400	—	>400
17–21	,, ...	400	550		650	600	600
	Diluent Limestone	5	3	4 (dried at 60°c)	5	3	5
22–25	Limestone/sulphur	400	400	—	500	350	500
26	,, ...	500	—	—	600	—	500
27	,, ...	650	—	600	—	—	—
27	,, (dried at 60°c)	350	—	300	—	—	—

TABLE 3—Sieve Analyses Specifications by Australian Fertilisers Ltd. (1958)

	% Sieve Analysis (B.S. Sieve Sizes, Converted from Original Tyler)						
	+10	−10 +18	−18 +25	−25 +72	−72 +100	−100	Fineness Factor F
Specified (Spec.)	0·9	9·6	19·8	37·8	14·4	17·5	0·408
Average (Av.)	0·9	26·1	17·9	33·1	10·7	11·3	0·314

Inflammability Tests

Although the Godbert (1934) apparatus is called an "inflammability" apparatus, it really measures explosion hazards. An additional test was, therefore, used for inflammability of bulk fertilisers. 1 g of material was placed on a fire brick, a small flame played on it for five seconds, and then the time was determined during which a flame was visible. (A darkened room was used, as a sulphur flame is not easy to see.) This test is not a standard test, but will give an approximate indication of ease of inflaming. Results are shown in Table 5. Duplicate tests always agreed to better than $\pm 20\%$, and the table also shows which of the samples melted during the test.

Rate of Travel of Flame

The test of Cohen and Luft (1955) was used. These workers determined the speed with which a flame travels over a heap of combustible material. This increases with combustion temperature and porosity, and decreases with melting point. Thus, while this test measures the spread of combustion it does not measure "inflammability" in the commonly understood sense. It gives coke and sugar a zero rating, coal an extremely low rating, sulphur and sawdust an intermediate, and magnesium a very high value.

The fertiliser mixtures were examined, and in no case was the flame observed to travel over the heap. Sulphur A showed a speed of flame travel of 0·9 cm/min, compared with 0·77 cm/min reported by Cohen and Luft (1955), while a 1 : 1 mixture of sulphur A and limestone 3 showed a speed of flame travel of 0·5 cm/min.

Electrostatic Discharge Hazard

Electrostatic discharges have been known to cause fires. Insulators can build up electrostatic charges, which leak away at a rate proportional to their conductivity. Resistivities were determined by clipping two copper electrodes of known dimensions over the sides of a small, dry beaker, which was then loosely filled with fertiliser. The resistance across the electrodes was measured with an insulation tester, and between each reading the empty beaker was checked to read infinity ($> 2 \times 10^{10}$ ohm). The results of the measurements are given in Table 6.

TABLE 4—Percent by Weight of Various Sulphur Fractions in a Number of Diluents, that Pass Half the Godbert Test on the Small Apparatus

Superphosphate		1	1 (Dried at 60°c)	2	2 (Dried at 60°c)
Sulphur A	as received	25	22	30	—
	−22 +60 mesh	40	32	40	—
	−60 +120 mesh	20	18	28	—
	−120 +240 mesh	10	—	10	—
	−240 mesh	5	—	6	—
Sulphur B	as received	23	—	26	—
	−22 +60 mesh	38	—	45	37
	−60 +120 mesh	18	—	17	—
	−120 +240 mesh	8	—	7	—
	−240 mesh	5	—	4	—
	Spec.	25	—	27	22
	Av.	30	—	33	27
Limestone		3	4 (dried at 60°c)	5	6
Sulphur A	as received	14	13	25	35
	−22 +60 mesh	20	23	35	40
	−60 +120 mesh	8	7½	20	20
	−120 +240 mesh	4	5	7½	10
	−240 mesh	2½	2	5	5
Sulphur B	as received	12	13	22	—
	−22 +60 mesh	23	23	35	—
	−60 +120 mesh	8	8	10	—
	−120 +240 mesh	4	4	6	—
	−240 mesh	2½	2	3	—
	Spec.	13	13	25	—
	Av.	17	20	30	—
Gypsum		7	8	9	
Sulphur A	as received	20	30	33	
	−22 +60 mesh	25	37	40	
	−60 +120 mesh	10	23	30	
	−120 +240 mesh	5	9	15	
	−240 mesh	3	6	7	
Sulphur B	as received	—	25	—	
	−22 +60 mesh	—	37	—	
	−60 +120 mesh	—	17	—	
	−120 +240 mesh	—	7½	—	
	−240 mesh	—	5	—	
	Spec.	—	27	—	
	Av.	—	30	—	

TABLE 5—Length of Time a Flame is Visible on a 1 g Sample of Fertiliser

Sample No.		As Received	− 22 Mesh
	Sulphurised Superphosphate		
10	400 lb S/ton	17 sec	4 sec
11	600	28	5
12	700	35	12
13	800	52 (melts)	32
14	224	4	—
15	400	15	4
16	224	7	—
17	400 (+ 6 lb cobalt)	8	—
18	400 (serpentine super)	4	4
19	224	5	4
20	400	8	5
21	800	65 (melts)	75 (melts)
	Limestone/Sulphur		
22	2 : 1	54 (melts)	25
23	600 lb S/ton	15	16
24	700	32	18
25	800	60 (melts)	28
26	600	28	12
27	600	25	—
A	Sulphur	90 (melts)	80 (melts)

TABLE 6—Resistivity of Fertiliser Mixtures

Sample No.		% Moisture Content	Resistivity (ohm-cm)
A	Sulphur	0·0	$> 10^{11}$
11	Sulphurised superphosphate	2·0	4×10^{8}
13	Sulphurised superphosphate	1·5	2×10^{9}
17	Sulphurised superphosphate	2·2	2×10^{6}
21	Sulphurised superphosphate	2·0	4×10^{6}
23	Limestone/sulphur	0·5	10^{9}
25	Limestone/sulphur	0·5	3×10^{9}
26	Limestone/sulphur	2·7	4×10^{7}
8	Gypsum	0·3	10^{8}
9	Gypsum	0·2	10^{8}

DISCUSSION

A number of interesting points arise from the tests:

The particle size of sulphur and diluent are of great importance in determining explosion limits. Following the method of Mason and Wheeler (1936a) a "fineness factor", F, has been calculated, which is the sum of the product of relative surface areas and the proportion present in each fraction. Table 1 gives F values for all sulphur samples and diluents. Mason and Wheeler (1936a) assumed that the specific surface of a dust

particle is inversely proportional to its mean diameter, and that the average mean diameter of the particles in a sieved fraction is the mean of the apertures of the two meshes between which the fractions lie. The surface area of the — 120 + 240 sieve fraction is taken to be 1·00 and all other surface areas are expressed relative to it. The — 240 fraction is assumed to have a relative surface area of 2·00.

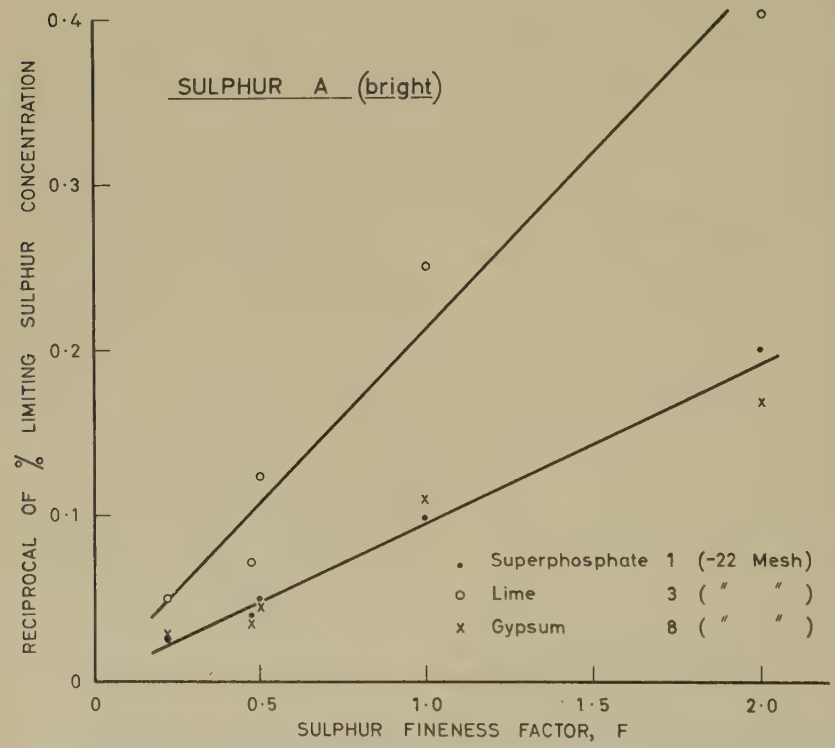

FIG. 1—Relationship between fineness factor and limiting concentration of Bright Sulphur A.

Figures 1 and 2 show plots of F for sulphur samples and sieved sulphur fractions against the reciprocal of percentage limiting sulphur concentration (given in Table 4) which is proportional to the quantity of just non-explosive mixture containing unit quantity of sulphur. The straight line graphs show that the specific surface of sulphur is directly proportional to the limiting sulphur concentration. As an example, — 240 mesh sulphur is about ten times as explosive as — 22 + 60 mesh sulphur, using the same diluent.

The specific surface of diluents also influences their damping properties considerably. Limestone 3 (F = 0·378), for example, is only about half

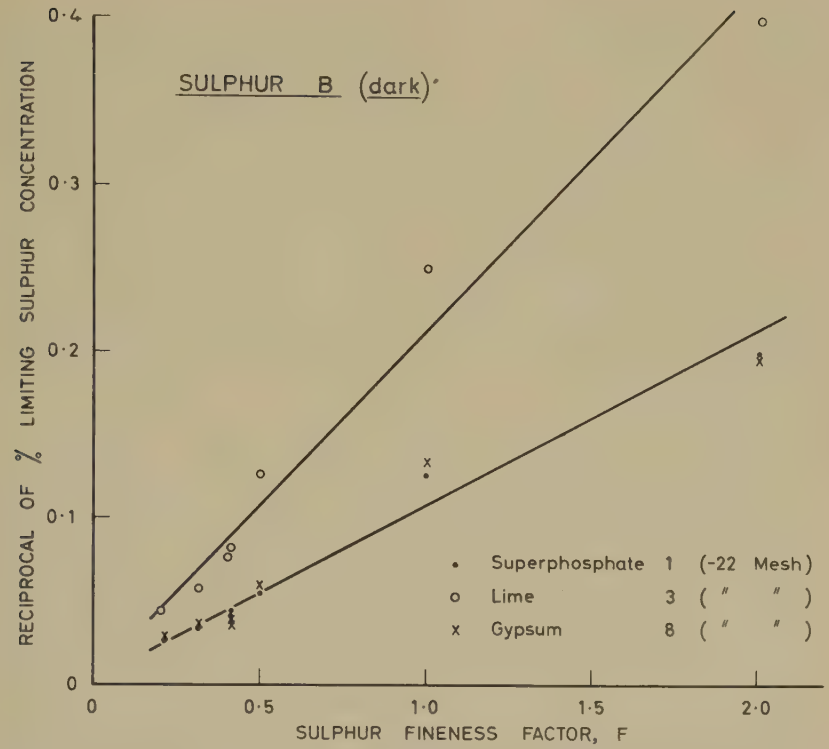

FIG. 2—Relationship between fineness factor and limiting concentration of Dark Sulphur B.

as effective as limestone 6 (F = 1·500). However, the efficiency of diluents increases at a slower rate than their surface areas, as is shown by the results in Table 4.

Superphosphate and gypsum of the same fineness factor are approximately equally effective in damping sulphur explosions, while limestone is decidedly less effective. With the + 22 sieve fraction removed, superphosphate 1 has F = 0·540, limestone 3 has F = 0·474 and gypsum 8 has F = 0·564. Results with these three commercial diluents, plotted in Figs 1 and 2 demonstrate the poorer damping ability of limestone. Table 4 shows that limestone 5 (F = 0·613) is still a poorer diluent than superphosphate 1, while finely ground limestone 6 (F = 1·500) approximately equals superphosphate 1. It should be noted that the original gypsum 8, with a small (11%) + 22 fraction, is probably a better diluent than superphosphate 1, containing 45% of + 22 particles. Godbert (1934) had shown that with a typical coal-dust, 35% limestone has the same damping power for combustion as 21% gypsum. Mason and Wheeler (1936b) gave similar figures and postulated that the water of crystallisation in gypsum (21%) gives it superior damping properties, as anhydrite (anhydrous gypsum) has only

the same damping power as limestone. Superphosphate contains about 5% water of crystallisation, as well as about 6% equilibrium water, and would, according to Mason and Wheeler's theory (1936b) be comparable with gypsum as a diluent.

Most of the mixtures received had low moisture contents, usually of the order that is normally in equilibrium with the atmosphere. However, limestone 4 contained 13·3% moisture, while mixture 27 (600 lb S per ton limestone) contained 11·5% moisture. Mixture 27 would certainly dry out on exposure to the atmosphere and Table 2 shows the large increase in explosion danger when it is dried at 60°C. Table 4 gives a few values which indicate that superphosphate dried at 60°C, when it had lost its equilibrium water, was a distinctly poorer damper than before drying. It is certainly considered dangerous to rely on any moisture in excess of equilibrium water which evaporates on storage, to reduce explosion risks of fertiliser-mixtures.

Australian Fertilisers (1958) suggest that dark sulphur is less dangerous than bright sulphur, in mixtures used for aerial spreading. In the present work explosion tests were only done on two samples, one bright (A) and one dark (B). The equal slopes of corresponding lines in Figs. 1 and 2 show that there is no significant difference in explosion risk of similar particle sizes of the two sulphur varieties. However, in the sieve analyses given by Australian Fertilisers (1958), dark sulphurs happen to be significantly coarser than bright sulphurs, while the few sieve analyses obtained here do not suggest the same applies for sulphurs obtained in New Zealand.

Table 5 shows the times that a 1 g sample of fertiliser stays alight after a small flame has played on it. This test has not been standardised, but it can easily be seen that using either superphosphate or limestone as a diluent, the length of burning increases rapidly beyond 500–600 lb/ton, suggesting that such mixtures are easily ignited. The samples, as received, burn rather longer than the — 22 mesh fraction, showing that coarser particles burn more easily, due to better access of air. The fineness of samples tends to have opposite effects on explosibility, although a fire could easily cause an explosion in a dust-cloud.

The test according to Cohen and Luft (1955) showed that in none of the mixtures tested here was a flame likely to travel over a heap of fertiliser. Only mixtures containing about 50% sulphur actually propagated a flame. In a small topdressing aircraft, however, even a non-spreading fire must be considered dangerous, as the cargo cannot easily be approached during flight.

The danger from electrostatic discharge is sometimes mentioned. Cox and Peace (1948) describe the ignition of dust-clouds by electrostatic discharge, and show that it is possible whenever there is much movement between non-conducting or poorly earthed components. Cooper (1953) discusses the practical estimation of electrostatic hazards, and concludes that only if the bulk resistivity of a charged mass exceeds 10^{11} ohm-cm, is there any real danger, while if the resistivity is below 10^{9} ohm-cm, the

material is virtually a perfect conductor for the present purpose. Elemental sulphur has a resistivity of about 10^{17} ohm-cm, and must, therefore, be considered a great electrostatic risk.

Table 6 indicates that loosely-packed fertiliser mixtures show great variations in resistivities. These must be strongly affected by moisture content and, in the case of superphosphate, by any free sulphuric acid present. It is evident, however, that in no case, other than that of free sulphur, is the resistivity near the danger limit of 10^{11} ohm–cm, so that all the fertiliser mixures examined here can be considered free from electrostatic discharge hazards.

The tests described here show that some of the sulphur-containing mixtures which have been applied from aircraft in New Zealand must be considered unsafe. This danger could be overcome either by stipulating that only relatively coarse sulphur must be used, or by simply limiting the amount of elemental sulphur in mixtures.

When fertilisers for sulphur-deficiency are formulated, a number of other aspects, such as speed of response (which increases with decreasing particle size), relation to phosphate-deficiency and cost of materials and aerial-topdressing charges, must be taken into consideration. When this is done, and using the results obtained here, it would seem desirable that a limit of 500 lb of commercially screened sulphur per ton of mixture be instituted (23% sulphur by weight) relying on the fact that commercial sulphur and diluent samples will not vary greatly in sieve analysis. It is considered that this limit is the maximum that provides an adequate inflammability safety margin for mixtures of sulphur in superphosphate or gypsum, but may very well be close to the danger point for mixtures with dry limestone.

In very sulphur-deficient areas with no phosphate response, it appears to be best to use commercial gypsum as a diluent, while in most areas superphosphate is far the best diluent, as it supplies sulphate and phosphate at the same time. Limestone is considered an unsuitable diluent, as it is less efficient in preventing explosions and supplies neither sulphate nor phosphate.

CONCLUSIONS

1. Because of explosion danger, fertilisers for aerial topdressing should be limited to a content of 500 lb commercial screened sulphur per ton of mixture. No electrostatic discharge risk is involved in the use of sulphur-containing fertilisers.
2. Superphosphate is the most suitable diluent for elemental sulphur. Gypsum could be used in very sulphur-deficient areas, while limestone is considered an unsuitable diluent.

ACKNOWLEDGMENTS

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AN INVESTIGATION OF THE FINE FRACTION OF SOME ROCK AND SOIL MATERIALS FROM ANTARCTICA

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(Received for publication, 1 May 1961)

Summary

The clay mineral content of some Beacon Sandstone samples is described. These rocks appear to have been derived by erosion of schist or similar metamorphic rock under arid climate. Talc in fine material of soil from Skelton Glacier moraine is probably derived by physical comminution rather than chemical weathering.

Introduction

Fine particle size materials have been found in various parts of Antarctica and have been investigated by several workers (Jensen, 1916; Blakemore and Swindale, 1958).

Some of the material found seems to be formed by weathering in place, but a large proportion of the finer materials are moraines, sand dune, or other transported material. Most of this transported material is derived from the physical decomposition of older rocks and the mineralogy of these materials would be related to that of the source rocks.

The most common sedimentary rock in Victoria Land is the Beacon Sandstone, a composite rock consisting largely of quartz sandstones, siltstones, claystones, and conglomerates (McKelvey and Webb 1961). It would appear likely that the matrix of these sediments is the origin of a large proportion of the fines found in the so-called soils of Antarctica. In order to elucidate this a number of samples of the Beacon Sandstone were analysed to determine the nature of the matrix material.

The samples P.C. 310, and P.C. 365–370 were all Beacon Sandstones from the Upper Taylor Glacier. Other samples were:

P.C. 392 "soil" from Stepaside Spur, Skelton Glacier.
P.C. 393 "soil" from Twin Rocks, Skelton Glacier.

The Beacon Sandstone samples were supplied by Mr P. N. Webb, of the Victoria University of Wellington, and the "soil" samples by Dr T. Hatherton, Geophysics Division of D.S.I.R. The sedimentary petrography of these Beacon Sandstone samples and the petrography and stratigraphy of the Beacon Sandstone (Group) is described by Webb (1960).

Methods

Some of the more indurated sandstone samples were crushed before analysis in order to release the fine fractions; others were broken down by water alone. Determinations on the Beacon Sandstone samples were made

of the iron oxides extracted by a dithionite-citrate reagent (Aguilera and Jackson, 1953), of silica and alumina extracted by dilute sodium hydroxide (Foster, 1953) and mechanical analysis by a method involving sedimentation and weighing of separate fractions. Clay mineral determinations on all samples were made by X-ray powder diffraction using the standard techniques of orientation of the basal spacing, saturation with magnesium and potassium ions, glycerol solvation and heat treatments. Silt fractions were examined as a dry powder.

RESULTS

Particle size determinations on the Beacon Sandstone samples and their Fe_2O_3, SiO_2 and Al_2O_3 contents are set out in the following table.

	Sand	Silt	Clay	Fe_2O_3	SiO_2	Al_2O_3
P.C. 364	79	16	5	0·2	0·74	0·3
P.C. 365	82	12	6	0·2	0·9	0·2
P.C. 366	84	8	7	0·7	1·0	0·2
P.C. 367	71	19	10	0·3	1·1	0·2
P.C. 368	91	5	2	1·1	0·3	0·2
P.C. 369	99	0·6	0·3	0·1	0·1	0·16
P.C. 370	95	3	2	0·05	0·6	0·16

Because of the crushing that some of the samples were subjected to, these figures are not an accurate measure of the particle size distribution but they indicate the approximate amount of fine material in the matrix. The iron oxide figure includes all the amorphous and microcrystalline iron oxides present in the matrix. This figure is high for P.C. 366 which is red and presumably contains some haematite, but is even higher for P.C. 368 which is a green orthoquartzite. Silica and alumina figures extracted by sodium hydroxide represent the amorphous fractions only. A little alumina has been dissolved, but the amounts are fairly constant. Silica varies markedly from sample to sample, but there is a rough correlation between the amount of silica and the amount of fine material. It appears that the silica extracted by this method is only the amorphous silica associated with the clay minerals and low values in samples P.C. 368, 369, 370, orthoquartzites may be due to recrystallisation of authigenic silica (Webb – in press).

Clay Mineralogy

The clay fractions were analysed by X-ray diffraction, with the following results:

P.C. 310 Mica, very well crystallised.
P.C. 364 Mica.
P.C. 365 Mica, with a trace of quartz.
P.C. 366 Mica, with a trace of chlorite and quartz.
P.C. 367 Mica, quartz and chlorite.
P.C. 368 Mica and a trace of chlorite.
P.C. 369 Mica, feldspar and some chlorite.
P.C. 370 Mica.
P.C. 392 Dominantly hydrated mica (partially and completely collapsing) and talc.
P.C. 393 Vermiculite, mica, and chlorite with a little quartz and feldspar.

With the exception of the last two samples, the samples are all from the Beacon Sandstone. The dominant mineral is mica, a well crystallised muscovite. In some of the samples chlorite is also present; in P.C. 367, there is a considerable amount.

X-ray analysis of some of the silt fractions gave the following results:

P.C. 364—Mica, quartz and feldspar.
P.C. 365 Mica, quartz and a little feldspar.
P.C. 366 Mica, chlorite, quartz and feldspar.
P.C. 367 Mica, chlorite, quartz and a trace of feldspar.
P.C. 368 Mica, chlorite, quartz and a trace of feldspar.
P.C. 369 Quartz and feldspar with a rather more weathered mica.
P.C. 370 Mica and quartz.

CONCLUSIONS

Quartz and feldspar appear in most silt fractions, but feldspar is not always present. The X-ray techniques used do not permit identification of the feldspar. Mica is present in all of these silts as well as in the clays; chlorite is also present in the silts when present in the clay fraction.

These results indicate that the matrix of the sandstone contains a little clay, sometimes as much as 10% cemented by small amounts of oxides of iron, aluminium, and silicon. The clay mineral is well crystallised muscovite, better crystallised than is usual for a soil mica, except in soils such as the weakly weathered soils of Central Otago, New Zealand, derived from micaceous rock. Chlorite is also present and may be responsible for the green colour observed in some of the sediments such as P.C. 366 and 367, green orthoquartz.

From the above evidence it would seem that the Beacon Sandstone is derived from the erosion of a schist or similar metamorphic rock under an arid climate that would not permit much hydration of the mica. Weathering was sufficient to remove most of the chlorite, and in most of the rocks only traces remain. If the micas had formed after deposition, from dehydration of other clay minerals, they would not be expected to extend into the silt fraction, but as both chlorite and mica are found in this particle size range they must have been deposited as silts as well as clays.

Any Quaternary moraine or other accumulation of fine debris would also be expected to contain micas and chlorites, derived from the sandstone, and evidence of chemical weathering would have to be drawn from the observation of the alteration or hydration of these minerals. Before this can be done with certainty, it is necessary to investigate other rocks which might give rise to clay minerals upon physical decomposition. Some evidence is provided by the two samples from the Skelton Glacier, one of which contains minerals, normally found in soils formed in temperate climates, which could have been derived from the rock or from weathering. The other contains talc, which is not usually found in soils and indicates that these materials are derived from physical comminution of rocks.

Blakemore and Swindale (1958) in analysing a soil from Scott Base, found that the clay fraction contained dioctahedral mica (muscovite),

interstratified (swelling and non-swelling) micaceous minerals, kaolin, quartz, and feldspar, an assemblage which would not be likely in soil derived from basalt, the rock type around Scott Base. These authors assume that all of this material is derived from erosion of such rocks as the Beacon Sandstone. This work shows that the sandstone contains most of the minerals found in the sample from Scott Base and confirms their assumption as to the origin of the clays.

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SOILS OF STEPHENS ISLAND

By W. T. WARD, Soil Bureau, Department of Scientific and Industrial Research, Wellington.

(Received for publication, 8 September 1960)

Summary

Morphological and chemical properties of seven soils occurring on Stephens Island (370 acres) are described. Two soils, which together occupy 90 acres, are extensively burrowed by nesting sea birds whose effect is discussed with reference to the process of soil formation. The two soils are extremely acid and possess a very high content of citric-soluble phosphorus and a very small content of exchangeable cations. It is inferred that manuring by sea birds makes the soils acid and enriches them in nitrogen and phosphorus. These effects are accompanied at first by enrichment of bases but intense leaching occurs as the pH falls.

INTRODUCTION

Stephens Island (40° 40′ S, 174° 00′ E) lies 2 miles off the northern coast of D'Urville Island and 58 miles north-west of Wellington. It is bounded on all sides by very steep cliffs which rise to 200 ft in the south and 900 ft in the west where the coast is exposed to the full force of the north-west gales that frequently sweep through Cook Strait. Although it has a very small area (370 acres) the island rises to 928 ft where a number of prominent ridges intersect near the western cliffs. The valleys between these ridges lack permanent streams and descend steeply to the edge of the cliffs where they each terminate in a V shaped notch. The land above the cliffs has an area of 200 acres and is mostly rough and broken, with slopes of 12°–38°. Easy land is of very small extent, occurring only at ridge crests. The island provides grazing for a few sheep and milking cows, and sanctuary for innumerable sea-birds.

Access to the island is by sea, and stores and mail are brought regularly from French Pass to the lighthouse keepers who, with their families, are the only residents. From the landing stage on the eastern cliffs a walking track leads to the houses situated in the north. A cable tramway (with two lifts) is used to convey stores, and from the upper winch house at 600 ft the tramline follows the contour past a disused naval barracks and past the keepers' houses, ending at the lighthouse. As there is no surface water on the island, residents and their stock depend on roof catchments for a supply.

Rainfall records show that the average annual rainfall, distributed evenly throughout the year, is 32·4 in. (100 raindays). Frosts are rarely experienced and the estimated mean annual temperature is 54°F. There are few days without wind but to some extent shelter is given from the north-west winds by the western cliffs which deflect their main force. When the wind is in this quarter, pockets of almost calm air lie in the lee of the cliffs and the higher parts of the island are enveloped in

cloud formed in the moisture-laden air as it rises from sea level to 900 ft. Similar shelter is not given by the low eastern cliffs and consequently winds which damage vegetation and buildings generally come from the south or south-east.

The rocks that form the island consist of indurated sandstones, muddy sandstones, and siltstones of the Maitai series (Dr H. W. Wellman, pers. comm.) and they dip at a very steep angle. Their strike is north-east and, on cliffs which follow the same direction, ledges and bluffs which interrupt the downward movement of talus are formed. Rock weathering begins with a loosening along joint planes and is followed by decay of the loosened fragments, but some of the fine-grained rocks with few joints resist weathering and contribute rounded boulders to the soil mantle. Midway between the upper and lower winchhouses a small area of unconsolidated sand unconformably overlies the Maitai rocks.

Early accounts of the island describe it as covered by low coastal forest dominated by kohekohe (*Dysoxylum spectabile*) with taupata (*Coprosma repens*), mahoe (*Melicytus ramiflorus*) and ngaio (*Myoporum laetum*), the whole interspersed with scattered nikau palms (*Rhopalostylis sapida*). Where vegetation was able to establish on the cliffs, and on the stony exposed ridges and knolls, the forest gave way to windswept, scrubby taupata, ngaio, and kowhai (*Edwardsia microphylla*).

The forest is now reduced to a few small and scattered remnants. Its destruction appears to date from 1894, when the lighthouse was built and access tracks and clearings for pasture were cut. With the opening of the canopy the wind become more strongly felt in the forest, and clearings were rapidly extended – in 1918, for instance, a southerly gale destroyed much of the forest on the southern slopes. The dead timber which once lay thickly was collected for firewood and there is now little evidence of the former extent of the forest cover. There were also a number of fires in the early years which reduced the extent of the forest, and stock – sheep and cattle brought in for domestic supplies – have in the past caused considerable damage and helped to prevent regeneration.

In place of the forest appeared tussock grasses, mainly *Poa caespitosa,* and nowadays English grasses are interspersed with native grasses north and east of the tramway where pastures were first established.

As with many other small islands, Stephens Island is a refuge for sea-birds and much of it, including most of the steep southern slopes and the sheltered northern valleys, is used as a breeding ground. There are also large numbers of a reptile, the tuatara (*Sphenodon punctatus*), somewhat smaller numbers of skinks and geckos, and a rare frog (*Liopelma hamiltoni*) which lives in a tumbled heap of boulders locally called the "frog-bank". At night many of the sea-birds occupy burrows in the soil and in many places these are so numerous that walking is hazardous, the soil collapsing at each step. The tuataras are active at night; during the daylight hours they mostly live in crevices among rocky outcrops and under logs but many occupy burrows excavated previously by the birds. The tuatara deposits its eggs in a shallow depression scraped in the topsoil. The eggs are then covered over with soil and dead leaves and left to hatch without further attention.

From time to time and particularly since 1920 there has been much interest in the protection and preservation of the native fauna and flora. To this end the Marine Department has virtually exterminated hawks and cats, reduced the numbers of stock (at one time there were 400 sheep on the island) and have fenced off the larger remnants of forest and scrub. Access of stock to the southern part of the island has been prevented since 1951 by a fence which extends north-west from a point near the landing stage.

In 1949, Dr W. H. Dawbin (Victoria University of Wellingon) began a field study of the tuatara and its environment, assisted by specialists in various fields. The soil survey, made in December 1954, is a contribution to this research.

Three soil series, named Ketu, Titahi, and Takaporewa soils, were distinguished. Ketu and Takaporewa soils are both developed on the Maitai rocks but the Takaporewa soils have been extensively influenced by seabirds. Titahi soils are developed on unconsolidated sands and occupy a very small area.

SOIL DESCRIPTIONS

The following seven soils are recognised (Fig. 1).

Ketu hill soil	1
Ketu hill soil, shallow phase	1a
Ketu hill soil, friable variant	1b
Ketu steepland soils	1c
Titahi hill soils	2
Takaporewa hill soil	3
Takaporewa steepland soil	3a

Ketu soils are developed on the weathered "Maitai" rocks – sandstones, muddy sandstones, and siltstones. In most places they occur together in a complex fashion, Ketu hill soil occupying slopes facing north, the shallow phase tending to be confined to ridges and to areas adjacent to rock outcrops, and the friable variant to the moist southern aspects. In spite of their close association in the field, the hill soils must be classified separately according to the genetic classification of New Zealand soils (Taylor, 1948; Taylor and Cox, 1956). As the climatic data suggest, the island lies near the boundary between the two zonal groups, the yellow-grey earths and the yellow-brown earths, and in consequence the aspect exerts considerable effect on soil morphology. Thus, on the sunny, somewhat arid sites occupied by Ketu hill soil features of yellow-grey earths (compact subsoil, mottling in B and C horizons) are observed. The friable variant, on the other hand, lacks these features and more nearly resembles a yellow-brown earth.

Ketu hill soil (1) occurs at the naval barracks and with other Ketu soils on the higher ridges of the central part of the island and near the winch-houses. It ranges in depth from 18 to 24 in, and has a thin sandy topsoil

with, commonly, a faint purplish hue, and a firm, almost structureless subsoil that in weathered sections has a coarse blocky appearance (Fig. 2). In many respects the soil resembles the Paremata soils which are developed on the mainland near Wellington.

The soil texture varies from place to place according to the nature of the underlying rock and as a result of mixing of parent materials by soil creep. The following profile from near the barracks (slope 24°; vegetation – *Poa caespitosa* with ryegrass, goosegrass and cocksfoot) is representative:

1 in. greyish brown (2·5Y 5/2) sandy loam; very friable; weakly developed fine granular structure; abundant roots; distinct boundary;

3 in. greyish brown to brown (10YR 5/2–3) sandy loam; friable; moderately developed fine and very fine granular structure; abundant roots; distinct boundary;

7 in. pale yellowish brown (10YR 6/4) heavy sandy loam; firm; weakly developed medium nutty structure; few distinct brownish yellow mottles; many roots; indistinct boundary.

10 in. yellowish brown (10YR 5/4) sandy clay loam; very firm; very weakly developed coarse blocky structure; with faint humus stains between blocks; few distinct fine and medium strong brown mottles; very few roots; indistinct boundary;

on very pale brown weathered fine sandstone.

In this profile a thin, discontinuous, reddish brown iron pan was observed at a depth of 27 in. This pan occurs in other places but is not everywhere present. Many bleached grains of sand may be seen in the upper two horizons. Samples (No. 6661A-D) were taken at this locality for analysis and the results are given in Table 1 which includes for comparison the analysis of a soil (5641A-C) from a comparable site at Ketu Bay, on the mainland to the south. The low pH of the Stephens Island sample, the higher content of citric-soluble phosphorus and the high level of nitrogen relative to carbon, are notable.

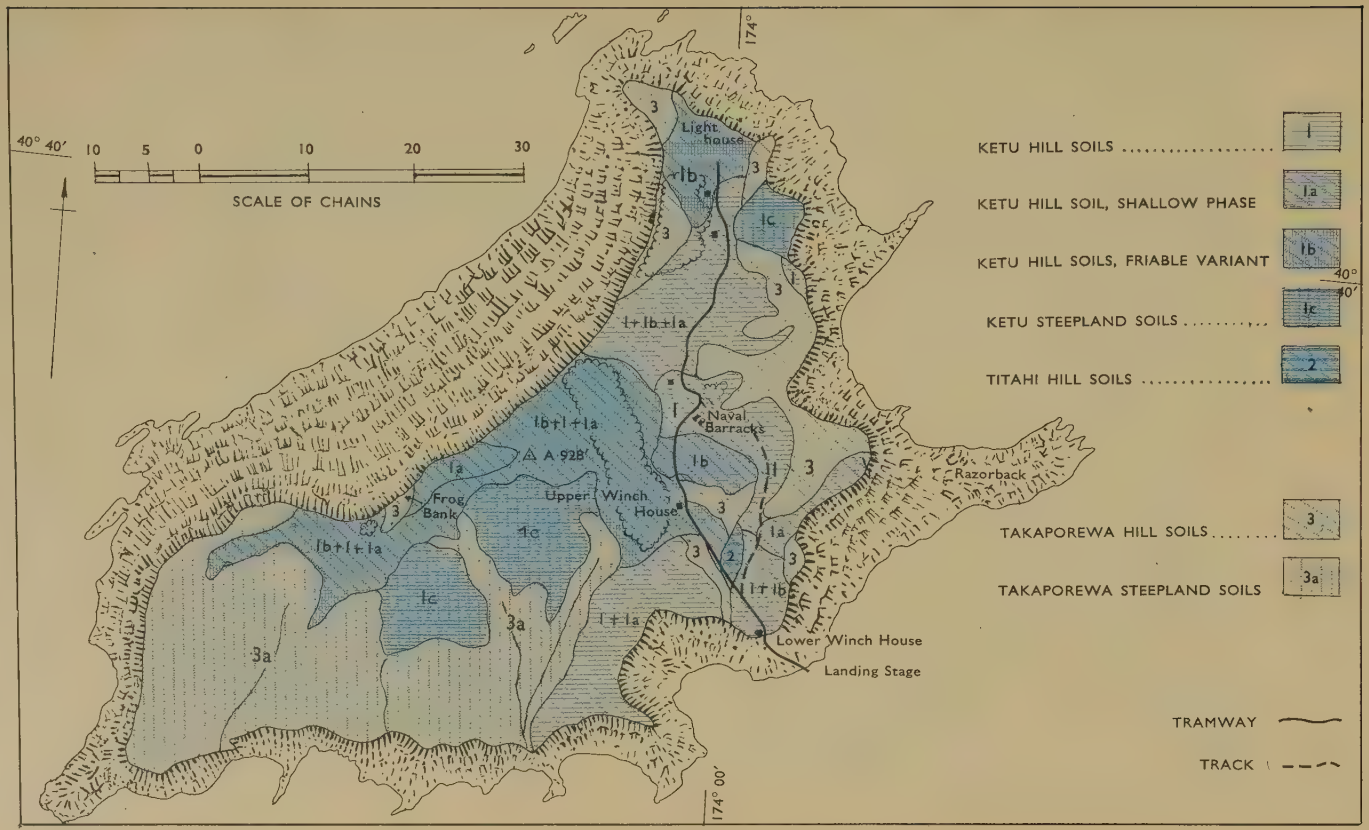
Ketu hill soil, shallow phase (1a) lies mostly on the ridge north of the lower winchhouse and to the west of the trig., but there are also many areas of the soil too small to show separately on the map, in the central part of the island. In most places rock lies close to the surface and bouldery outcrops are common. The topsoil is thin, and stony in many places, and where the soil is very shallow the subsoil horizon is absent. Where the soil is deepest it closely resembles Ketu hill soil but lacks mottling in the subsoil. Its boundaries with Ketu hill soil are ill-defined.

On sunny faces at low levels the soil retains little moisture and supports only the hardy barley grass (*Hordeum marinum*), *Orysopsis miliacea,* and scattered patches of unthrifty ryegrass. A profile from a slope of 8° on the ridge leading to the Razorback is:

4 in. dark brown (10YR 3/3) silt loam; very friable; moderately developed fine granular structure; abundant roots; distinct boundary;

8 in. pale yellowish brown (10YR 6/4) stony loam; firm; weakly developed nutty structure; few roots; indistinct boundary.

on brownish yellow (10YR 6/6) very stony loam overlying unweathered rock at 20 in.



S.B. 728 S

FIG. 1—Soil map of Stephens Island

FIG. 2—Ketu hill soil. The hammer is 9 in. in length.

TABLE 1—Analyses by Moira Young and J. C. Jennings.

—	Depth (in.)	pH	P* mg %	C %	N %	C/N	CEC me %	TEB me %	BS %	Exchangeable Cations (me %)				Ca/Mg	Na/TEB	Lab. No.
										Ca	Mg	K	Na			
Ketu hill soil; Ketu Bay, Pelorus Sound	0–4	5·4	3	3·9	0·29	13	19·7	9·3	47	5·4	4·0	0·95	0·50	1·35	5	5641A
	6–12	5·5	1				16·4	7·9	48	4·1	3·9	0·70	0·65	1·05	8	B
	12–30	5·3	0·5				19·1	5·7	30	2·3	2·8	0·65	0·60	0·82	11	C
Ketu hill soil; Stephens Island	0–2	4·9	6	1·3	0·25	5	9·1	4·5	49	1·9	1·7	0·35	0·5	1·12	11	6661A
	2–6	4·9	14	0·7	0·11	6	9·4	4·3	46	1·7	1·5	0·40	0·5	1·13	11	B
	6–11	4·8	12	0·8	0·12	7	12·2	2·8	23	1·1	0·9	0·15	0·6	1·22	21	C
	11–21	4·8	4	0·5	0·07	7	10·9	2·3	21	0·9	0·8	0·10	0·9	1·13	39	D
Ketu hill soil, friable variant; Stephens Island	0–2	5·5	39	8·1	1·05	8	35·2	34·6	98	21·6	5·2	2·45	1·7	4·15	5	6660A
	2–7	5·6	14	1·3	0·21	6	13·6	9·9	73	5·7	3·0	1·20	0·6	1·90	6	B
	7–14	6·4	5	0·6	0·12	5	10·0	9·3	93	4·4	3·4	1·00	0·6	1·29	6	C
	16–24	6·5	3	0·4	0·11	4	9·1	7·9	80	3·3	3·2	0·85	0·7	1·03	9	D
Titahi hill soils; Stephens Island	0–6	3·9	76	4·9	0·54	9	22·1	1·8	8	1·4	0·7	0·20	0·3	2·0	17	6662A
	6–18	3·8	54	1·2	0·14	9	11·1	0·5	5	0·4	0·1	0·10	0·2	1·0	40	B
	18–33	4·1	67	0·6	0·08	8	10·9	0·8	7	0·2	0·4	0·10	0·3	1·0	37	C
Titahi hill soils; Plimmerton	0–7	5·7	1	3·8	0·22	17	10·8	5·2	48	3·3	2·3	0·6		1·43	—	4147A
	10–15	5·3	1	1·1	0·08	14	7·1	2·8	39	1·7	1·5	0·5		1·13	—	B
Takaporewa hill soil; Stephens Island	0–6	3·8	201	8·3	0·76	11	38·0	5·3	14	3·0	0·8	0·70	0·7	3·75	13	6663A
	6–12	3·0	181	6·8	0·60	11	39·6	0·9	2	0·2	0·0	0·65	0·3	—	33	B
	12–18	3·0	191	5·9	0·45	13	39·1	0·9	2	0·2	0·2	0·85	0·5	1·0	55	C
	18–24	3·0	191	0·7	0·13	5	19·5	0·7	4	0·1	0·0	0·55	0·1	—	14	D

*Soluble in 1% citric acid.

At high levels pastures are more vigorous because evaporation is less rapid and cooler temperatures are experienced. With increasing elevation the soil becomes paler in colour and its horizons become somewhat less well marked. These features are illustrated in the following profile from a slope of 14° near the frogbank (vegetation – barley grass, ryegrass, tall fescue, Yorkshire fog, cocksfoot).

4 in. brown (10YR 5/3) silt loam; firm; moderately developed medium and fine nutty and cast granular structure; many roots; diffuse boundary;
on brownish yellow (10YR 6/6) stony loam; very firm; very weakly developed medium nutty structure; few roots.

*Ketu hill soil, friable variant** (1b) occupies slopes of 12–28° which, especially at low levels, face the south. It has silty textures and differs from Ketu hill soil in that its subsoil is friable. Topsoil and subsoil horizons grade easily into one another and in weathered cuttings the characteristic blocky subsoil structures of Ketu hill soil are not found.

A typical profile under forest (ngaio, kawakawa, mahoe) near the lighthouse (slope 14°) is:

1 in. very dark greyish brown (10YR 3/2) silt loam; very friable; moderately developed fine and medium granular structure; few bleached sand grains; distinct boundary;
5 in. brown (10YR 4/3) silt loam; friable; moderately developed fine and medium nutty structure; many roots; diffuse boundary;
6 in. dark yellowish brown (10YR 4/4) silt loam; friable; weakly developed medium nutty structure; many roots; diffuse boundary;
on yellowish brown heavy silt loam; firm; massive; grading to weathered siltstone.

Under pasture the topmost horizon cannot be distinguished and the topsoil contains many well developed granular aggregates.

On shady sites the soil is paler in colour and in places fine dark reddish brown mottles occur in the subsoil. Here, pastures are of high quality, with white clover as a prominent constituent.

The results obtained by analysis of samples of the soil (No. 6660A-D) indicate a very high content of phosphorus and high level of nitrogen relative to carbon. The high percentage base saturation associated with a moderate pH is in marked contrast with the figures obtained for Ketu hill soil.

Ketu steepland soils (1c) lie on slopes of 30–36° at high levels south of the trig. They are shallow and profiles are poorly developed owing to the creep of soil downslope. In some places shallow slips expose weakly weathered rock and elsewhere rock outcrops interrupt the smooth slopes.

The soils support a variety of plants and there are many scattered clumps of trees and patches of sedge (*Carex forsteri*).

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*A soil variant is closely related to the soil series from which it takes its name but differs significantly in at least one differentiating characteristic. It is really a separate soil series, but is of too small an extent to justify the establishment of a new name. (Soil Survey Staff, 1951.)

A profile from a slope of 32° under taupata and ngaio with cocksfoot and *Poa caespitosa* is:

2 in. very dark greyish brown (10YR 3/2) silt loam; very friable; moderately developed fine granular structure; abundant fibrous roots; distinct boundary;

5 in. brown (10YR 4/3) silt loam; friable; moderately developed medium and fine nutty and granular structures; very many roots; distinct boundary;

on yellowish brown (10YR 5/6) heavy silt loam; very firm; massive.

In some places sheet erosion has stripped away the topmost horizon and in others both horizons are poorly developed because of active soil creep.

Where slopes of less than 43° occur on the cliffs surrounding the island there is a thin mantle of soil which varies in depth from 3–12 in. Soil creep is very active and the scars of debris slides are slow to regrass.

A profile from the eastern cliffs under ngaio scrub, Yorkshire fog, cocksfoot and *Poa caespitosa* (slope 38°) is:

4 in. dark greyish brown (10YR 4/2) gravelly loam; firm; weakly developed fine granular structure; abundant roots; indistinct boundary.

on weakly weathered siltstone.

For the most part, the deeper steepland soils resemble Ketu hill soil, friable variant, rather than Ketu hill soil.

Titahi hill soils (2) are developed on unconsolidated sands, possibly of holocene age, midway between the two winchhouses. The sands are not extensive and the soils occupy a very small area. They are friable, with granular and nutty structures and ill-defined horizons, and contain many burrows excavated by the birds. These burrows are unstable and liable to collapse under a heavy tread. Much loose sand thrown out of them lies on the surface and is blown about in strong winds. At present there is little wind erosion because of a good cover of native and English grasses but should this be destroyed, erosion of the soils would undoubtedly be rapid.

A profile from a slope of 14° near the tramway (vegetation – cocksfoot, ryegrass and *Poa caespitosa*) is:

6 in. dark brown (7·5YR 4/2) loamy sand; very friable; moderately developed medium and fine granular structure; many bleached sand grains; abundant roots; diffuse boundary;

12 in. dark brown (7·5YR 3/3) loamy sand; friable; weakly developed medium nutty and fine granular structures; many roots; diffuse boundary;

15 in. brown (7·5YR 5/4) loamy sand with faint pale yellowish brown worm casts; firm; structureless; few roots; diffuse boundary;

on yellowish brown (10YR 5/6) sand; firm; slightly compact; structureless.

A similar soil is widespread about Titahi Bay and Plimmerton on the Wellington coast and the analyses of samples from the latter locality (No. 4147A-B) is given together with some for samples taken from Stephens Island (No. 6662A-C). It is evident that the soil on the island is much more acid than that on the mainland and that it is much higher in phosphorus and nitrogen, but has a lower content of exchangeable cations.

Takaporewa soils have been so intensively burrowed by sea-birds* that in most profiles little trace remains of the soil horizons and structures that existed before burrowing. Of the profiles whose original nature can be identified with reasonable certainty the bulk were originally Ketu hill soil, friable variant. Only about one profile in four seems to have been Ketu hill soil. This apparent preference for the friable variant as a nesting site appears to result from the difference in structure between the hill soil and the friable variant, the latter being the more easy to dig. This view is supported by the fact that scrapings and short burrows which have the appearance of being abandoned are to be found in places on Ketu hill soil, and it is possible that birds, discouraged by the resistance offered by the firm subsoil, sought other sites for their burrows. The boundaries between the burrowed Takaporewa soils and the Ketu hill soils are sharply defined and there is no evidence to suggest that the nesting areas are being extended at the expense of one or the other soil.

Takaporewa hill soil (3) occurs in various places near the head of the cliffs in the northern part of the island and near the frogbank. It is exposed to the westerly winds only in one place, north-west of the lighthouse. The soil is dark brown to dark reddish brown in colour with a deep friable topsoil and a friable subsoil honeycombed with short burrows about 5 in. in diameter and 3–4 ft long. At the time of the survey, many burrows were inhabited by dove petrel (*Pachyptela turtur*) chicks (Fig. 3). The floors of the burrows consist of smoothed, trampled soil fragments damp with droppings. From time to time pieces of soil are dislodged from the unstable roof, building up the level of the floor and burying in it pieces of eggshells and leaves used to line the nest.† Occasionally, birds and chicks are buried by large falls of earth.

The following profile from near the upper winchhouse is typical (slope 24°, vegetation – tall fescue, ryegrass, Yorkshire fog):

8 in. brown (10YR 4/3) silt loam; friable; moderately developed fine and medium granular structure; many bleached sand grains; many roots; diffuse boundary;

5 in. dark reddish brown (5YR 3/3) silt loam; very friable; moderately developed fine granular structure; abundant bleached sand grains; few small pieces of fresh mudstone; many roots; diffuse boundary;

5 in. dark brown (7·5YR 4/4) silt loam; friable; weakly developed medium and fine granular and nutty structures; few roots; few burrows containing dove petrel chicks; indistinct boundary;

on yellowish brown (10YR 5/6) heavy silt loam; firm; massive, with pockets of granular aggregates in places; few roots.

The soil ranges in depth from 18 to 30 in. and in places the weathered parent material is stained brown or dark brown by humus. Samples taken

*The main species that inhabit burrows are the dove petrel (*Pachyptela turtur*) and muttonbird (*Puffineus griseus*). There are also a few little blue penguins (*Eudyptula minor*). Roosting birds, particularly the red-billed gull (*Larus novaezelandiae*) and the black-backed gull (*L. dominicanus*), are very common on the island. (W. H. Dawbin, pers. comm.)

†Material collected from the floor of a petrel burrow on Ocean Island (Auckland Islands) by Dr R. A. Falla had a pH of 3·3, 31 mg%P (1% citric acid soluble), 31·4%C, and 2·5%N (sample No. 6668).

FIG. 3—Takaporewa hill soil. The soil is friable, with a granular structure. The burrows in the lower part of the profile are each occupied by dove petrels.

for analysis (No. 6663A-D) were extremely acid, had a very high content of citric acid-soluble phosphorus and were very poorly supplied with exchangeable cations. The water-soluble salts (Table 2) are high in sodium and chlorine and contained nitrites (quantity not determined).

TABLE 2—Water-soluble Salts – Takaporewa Hill Soil

Depth (in.)	Ca me %	Mg me %	K me %	Na me %	Cl me %	NO_3 me %	Total me %
0–6	0·41	0·93	0·08	1·36	1·8	0·04	4·64
6–12	0·21	0·26	0·10	0·70	0·4	0·27	1·94
12–18	0·15	0·15	0·12	0·68	0·3	0·27	1·67
18–24	0·22	0·18	0·12	0·51	0·3	0·28	1·61

Takaporewa steepland soil (3a) occupies the steep slopes (30°–36°) lying above the southern cliffs, where it extends to about the 700 ft level. The soil varies in depth from 4–12 in. and in many places on ridges and valley sides bare rock is exposed. Burrows are particularly numerous and as they lie close to the surface the soil is treacherous and difficult to walk on.

A profile from a site (slope 36°) half a mile west of the landing stage is:

7 in. dark brown (7·5YR 3/2) silt loam; loose; moderately developed medium and fine granular structures; very many roots; distinct irregular boundary;

on very weakly weathered sandstone.

The vegetation consists almost wholly of *Poa caespitosa,* but in places there are patches of sedge (*Carex forsteri*) and scattered scrub-like ngaio trees. Between the tussocks the ground is bare, the surface worn smooth by the trampling of birds. At the base of each tussock there are large numbers of rounded and smoothed soil fragments which have been swept into these sheltered places by the birds as they pass to and fro, in the same manner as gravel is swept to the sides of a road by passing cars.

Because of the loose texture and steep slope, the erosion hazard is great and much damage would follow destruction of the plant cover.

DISCUSSION

The Organic Cycle

For their continued growth, plants and animals must obtain nutrient elements from the soil. On death, these are returned to the earth, where they may again be utilised by other organisms. This cycling of elements between soil, plant and animal is termed the "organic cycle" and is part of the general process of soil formation. It is most often visualised as operating through plants, the elements in the soil being absorbed by roots and returned to the soil when the leaves and branches fall and rot away.

As a vector in the organic cycle, the tuatara on Stephens Island excites no special comment. Its food consists mainly of beetles, wetas and snails that live on plant material. Its droppings, and ultimately the dead animal, merely return to the soil what was once taken from it. This is not the case with the sea-birds, which derive their nutriment from sea food and in their droppings and remains introduce new material to the soil system. In this respect, the birds supplement the organic cycle. Also worthy of note are their other activities of nesting and burrowing, which hasten in great degree the natural incorporation and decay of organic matter in the soil. Thus, the building of nests accelerates the return of plant material. It also leads to the concentration of organic matter in the nesting areas and causes its depletion in adjoining areas. Similarly, the excavation of burrows hastens the movement of soil usually accomplished by earthworms and their like and by soil creep. By a kind of subsoiling action, it "homogenises" the soil, so destroying the original horizons and preventing them from reforming. The chief effect of these activities on the physical structure of the soil is to round the original soil aggregates and to incorporate decomposing dung and vegetable material. As a consequence of these changes in structure and physical composition, the soils become porous, well aerated and well drained. In these ways the sea-birds play an important part in the process of soil formation on Stephens Island and, because of the extent of their effect on the organic cycle, the soils there differ markedly from those developed in similar environments on the mainland where bird colonies are absent.

Soil Chemistry

The continual manuring of the soils by birds inevitably alters the chemical constitution of the soil, particularly where densely populated colonies occur. As the birds have complete freedom of movement, all soils on Stephens Island have been manured to some degree. For this reason it is difficult to determine the quantitative changes that have occurred, although some indication of their magnitude is given by comparison of the Stephens Island soils with those of the mainland.

It is evident from the analyses in Table 1 that all of the soils on Stephens Island have high levels of nitrogen relative to carbon and because of this the carbon/nitrogen ratios are mostly low to very low. It should be noted that the Ketu soils are moderately to strongly acid, whereas the other soils, which have a much higher bird population (indicated by the number of burrows), are extremely acid. The content of 1% citric acid-soluble phosphorus rises with increasing numbers of birds. On the other hand, the level of base saturation ranges from moderate to very high in Ketu soils, which are not burrowed by the birds, and low to very low where birds are numerous.

It is inferred, therefore, that manuring is, at first, accompanied by a slight increase in acidity and by enrichment of nitrogen, phosphorus and exchangeable cations. With continued addition of manure at high rates the

phosphorus content increases and, with pH falling below 4–4·5, there is a reversal in the base saturation trend, and the soils become very strongly leached of cations.

Some features of the soils (the low calcium/magnesium ratios and the high exchangeable sodium content of some soils) are perhaps the effect of sea salt deposited directly on the island and not the result of the activities of the birds. The humus stains observed in places below the Takaporewa soils and the blocky nature of the subsoil in Ketu hill soil may, possibly, be due to this cause.

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ESTIMATION OF OPEN WATER EVAPORATION IN NEW ZEALAND

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Summary

An account is given of tank evaporation measurements in New Zealand, with particular reference to the errors involved, the reduction factors to open water, and the relation to meteorological conditions. A modified form of Penman's equation for calculating monthly tank evaporation from weather records has been found to agree satisfactorily with the observed tank evaporation, and it has been used as a basis for estimates of open water evaporation for a number of stations lacking evaporimeters. For most of the stations investigated (35 out of 41) the estimated annual open water evaporations lie between 24 and 36 inches. The highest estimated values of 37 to 44 inches have been obtained for stations in Marlborough, North Otago, Canterbury, and Hawke's Bay.

INTRODUCTION

The problem of estimating evaporation from reservoirs and lakes, sometimes called "open-water evaporation", may be approached in various ways:

(a) The most direct method is the water-budget method, in which evaporation is determined from measurements of inflow, outflow, and changes in storage. Unfortunately this method is not practicable for most lakes on account of the uncertainty of seepage.

(b) Measurement by means of a tank, at the surface of which the process of evaporation is considered to be somewhat similar to that from a reservoir or lake. In practice it is necessary to make a comparison with "open water" for each type of tank to determine the "reduction factor".

(c) There are several indirect methods; in particular, a method involving calculation of the available energy.

The only method used to any extent in New Zealand so far has been that using a tank. Measurements with tank evaporimeters have been made at about thirty stations, mainly during recent years. This investigation is concerned with the interpretation of these measurements, partly for estimating open-water evaporation, but to some extent also for estimating evaporation from pastures. The relation of tank evaporation to meteorological conditions is also discussed.

MEASUREMENT OF TANK EVAPORATION

Description of Tanks

NEW ZEALAND SUNKEN PAN EVAPORIMETER

The photograph (Fig. 1), of this evaporimeter illustrates most of the features of the sunken pan type hitherto in general use in New Zealand. It is 3 ft in diameter and 3 ft deep, and is buried in the ground with the

FIG. 1—Sunken pan evaporimeter at Kelburn, Wellington.

top of the rim 2 to 3 in. above the surface. The height of the water-level is indicated by means of a vertical rod supported by a glass float set in a stilling well to eliminate wave-motion. The level is measured by means of a micrometer wheel mounted on the side of the tank.

In most respects the New Zealand tank is similar to the standard Australian type. The New Zealand tank, however, is made of waterproofed concrete, whereas the Australian type is made of galvanised iron, and it is set in an outer tank of diameter four feet, which is filled with water to the rim.

NEW ZEALAND RAISED PAN EVAPORIMETER

The New Zealand raised pan evaporimeter (Fig. 2) was originally introduced by the Ministry of Works in the Canterbury Irrigation Schemes in 1936. It is virtually identical with the U.S. Weather Bureau Class A Evaporimeter, and consists of a galvanised iron tank 4 ft in diameter by 12 in. deep mounted on a wooden stand the top of which is 6 in. above the ground. This is regarded by the World Meteorological Organization as

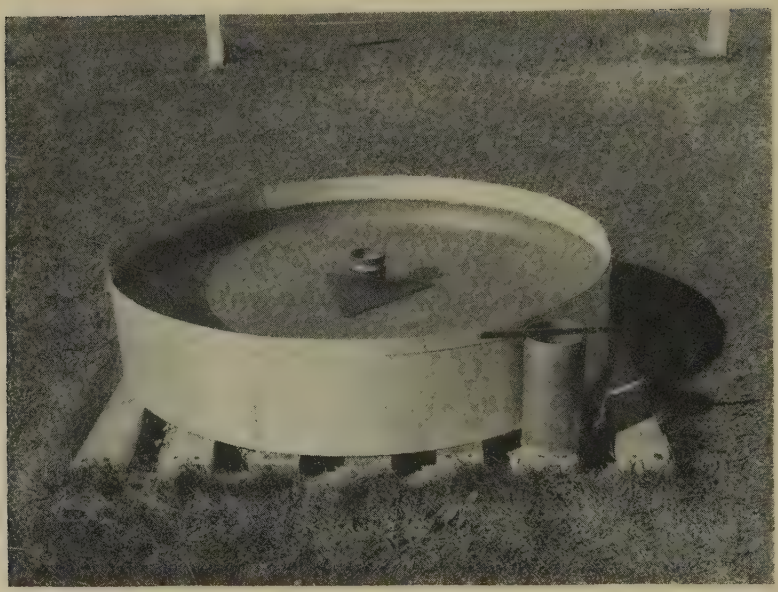

FIG. 2—Raised pan evaporimeter at Taieri aerodrome, Dunedin.

the standard type for the International Geophysical Year Programme. Nearly all new installations in New Zealand since 1956 have been of this type.

Errors in Measurement

LOOSENESS OR "BACKLASH" IN THE MICROMETER WHEEL

After a few years' use the thread of the micrometer screw commences to wear, and this causes uncertainty in the daily readings, so that if the instrument is read, screwed back to the normal position, and immediately read again, the difference in readings with a badly worn thread may amount to as much as ·02 in. Since the average daily winter evaporation at most stations is only ·03 in., this type of error frequently invalidates individual daily readings in winter. Monthly totals are not usually seriously affected, especially in summer.

VARIATIONS IN WATER LEVEL

Variations in water level are known to have a considerable effect on evaporation. In experiments by Bonython (1950) with a sunken pan evaporimeter, a lowering of the water level by 2 in. decreased the evaporation by 15%. In the raised pan evaporimeter a change of water level probably has less effect. (Ventikeshwaran, Jagannathan and Ramakishran, 1959.)

In the older New Zealand records the range of level allowed was rather too large. In the results presented here the average water level was about

4 in. below the rim of the tank. However, since December 1955 the level in the sunken pan evaporimeter has usually averaged 2½ in. below the rim, with a range of only 1 in. Comparisons with a Piche atmometer at Wellington before and after raising the water-level indicated that the change had increased evaporation from the tank by about 10%.

Errors due to Wind

In very windy conditions it is known that spray is blown out of the tank, causing the apparent evaporation to be too high.

Errors due to Rain

When appreciable rain is recorded, the assumption is made that the depth of rain in the tank is identical with that recorded by the raingauge, and the apparent evaporation is corrected accordingly. In general this assumption is not correct. The relative difference between the two catches is greatest in windy conditions.

By far the most serious errors in tank evaporation arise from these differences in catch. The errors were examined by Bilham (1932) for Valentia, in Ireland, and he stated that two different effects cause errors of opposite sign:

(i) With large drops there is considerable splash-out from the tank, which accordingly catches less than the raingauge. The apparent evaporation thus tends to be too large. This effect is often aggravated by simultaneous loss by wind.

(ii) With small drops, the tank (if it is a sunken pan) acts as a shielded raingauge, and in very windy conditions tends to catch more than the normally exposed gauge. On this account the apparent evaporation tends to be too small and in winter may even be negative.

A comparison of evaporation from a sunken pan evaporimeter with that from a Piche atmometer at Wellington (see Appendix) suggests that average annual totals and average monthly totals in summer are not seriously affected. However, when evaporation is lowest, from May to August, evaporation measurements are not very accurate.

Results of Tank Measurements

General

Table 1 gives average monthly and annual totals of tank evaporation for New Zealand stations having records for at least five years. For convenience, the altitudes of the stations and the annual rainfalls are also listed. The significance of the reduction factors is discussed in the next section. The location of the stations may be conveniently obtained from Fig. 3.

TABLE 1—Mean Monthly and Annual Tank Evaporation (in.)

R.F. = Reduction Factor to open-water with prevailing water level

NOTE: "Rainfall" refers to mean annual rainfall 1921–50.

Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Year
I. SUNKEN-PAN EVAPORIMETER, NEW ZEALAND TYPE (R.F. 0·87)												
Ruakura, Hamilton (1941–55) Alt. 131 ft Rainfall 46 in.												
4·9	3·8	3·4	2·0	1·5	1·3	1·1	1·5	2·0	2·8	3·8	4·4	32.5
Rukuhia, Hamilton (1950–55) Alt. 215 ft Rainfall 48 in.												
5·5	4·3	4·0	2·4	1·7	1·5	1·3	1·5	2·3	3·0	3·9	5·0	36·4
Taupo (1951–55) Alt. 1,221 ft Rainfall 44 in.												
5·6	3·9	3·7	2·2	1·3	1·1	1·0	1·3	2·1	3·1	3·7	4·7	33·7
Waerenga-O-Kuri (1948–55) Alt. 1130 ft Rainfall 51 in.												
4.3	3·3	3·2	2·1	1·5	1·4	1·3	1·4	2·0	2·9	3·4	4·4	31·2
Palmerston North (1941–55) Alt. 110 ft Rainfall 39 in.												
4·8	4·0	2·8	1·9	1·3	0·9	1·0	1·4	1·9	2·9	3·7	4·5	31·1
Kelburn, Wellington (1949–55) Alt. 415 ft Rainfall 49 in.												
4·8	3·7	3·3	2·1	1·4	1·1	0·8	1·3	1·9	2·7	3·6	4·3	31·0
Appleby, Nelson (1934–55) Alt. 57 ft Rainfall 37 in.												
5·3	4·1	3·6	2·2	1·3	0·9	0·9	1·2	2·0	2·6	4·1	5·3	33·7
Lake Grassmere (1945–54) Alt. 5 ft Rainfall 22 in.												
7·8	6·3	5·4	3·3	2·3	1·8	1·6	2·2	2·8	3·6	5·8	7·3	50·2
Lincoln College (1945–55) Alt. 36 ft Rainfall 26 in.												
5·9	5·0	3·8	2·1	1·3	0·8	0·8	1·5	2·6	3·4	4·7	5·2	37·2
Winchmore, Ashburton (1949–55) Alt. 526 ft Rainfall 29 in.												
5·3	4·4	3·5	2·4	1·4	0·9	0·9	1·2	2·3	3·4	4·9	4·9	35·5
Tara Hills, Omarama (1950–55) Alt. 1,600 ft Rainfall 21 in.												
7·7	5·6	4·8	2·6	1·2	0·4	0·4	1·0	2·7	4·4	5·9	6·8	43·5
Alexandra (1929–50) Alt. 520 ft Rainfall 13 in.												
5·3	4·1	3·3	1·7	0·8	0·3	0·2	0·9	1·8	3·1	4·2	5·0	30·6
Invercargill (1946–55) Alt. 8 ft Rainfall 43 in.												
4·9	3·7	2·9	1·6	0·9	0·5	0·6	0·9	1·7	2·8	3·9	4·3	28·6

TABLE 1—continued

Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Year
II. SUNKEN-PAN EVAPORIMETER, ELECTRICITY DEPARTMENT TYPE (R.F. 0·85)												
Onepoto, Lake Waikaremoana (1935–55) Alt. 2,100 ft Rainfall 77 in.												
4·4	3·5	2·8	1·6	0·9	0·7	0·6	0·9	1·6	2·7	3·3	4·4	..... 27·4
III. SUNKEN-PAN EVAPORIMETER, BRITISH TYPE (R.F. 0·95)												
Thorndon, Wellington (1917–27) Alt. 10 ft Rainfall 42 in.												
5.3	4·3	3·7	2·0	1·2	0·8	0·9	1·2	2·0	3·3	4·2	5·0	..... 33·9
IV. RAISED-PAN EVAPORIMETER (R.F. 0·72)												
Omaka, Blenheim (1937–53) Alt. 100 ft Rainfall 27 in.												
7·5	6·4	4·8	2·9	1·8	1·6	1·5	2·0	2·9	4·3	5·6	7·1	..... 48·5
Darfield, North Canterbury (1951–57) Alt. 641 ft Rainfall 31 in.												
6·1	4·4	3·1	2·0	1·0	0·9	0·8	1·1	2·3	3·4	4·4	5·0	..... 34·5
"Rudstone", Methven (1936–50) Alt. 1,217 ft Rainfall 42 in.												
6·4	5·3	4·5	2·8	2·3	1·6	1·6	2·3	3·3	4·9	5·6	5·2	..... 46·7
Pendarves, Kinkora (1936–47) Alt 120 ft Rainfall 28 in.												
6·2	4·7	3·5	2·0	1·4	1·0	0·7	1·2	2·6	3·7	4·9	5·3	..... 37·2
Hinds (1936–47) Alt. 400 ft Rainfall 29 in.												
5·6	4·5	2·9	2·1	1·6	1·2	0·9	1·7	2·8	3·9	4·7	5·3	..... 37·2

NOTE: Winter values at most inland stations in Canterbury and Otago are affected by the freezing-over of the tank.

Detailed discussion of the relationship with other meteorological data and of the space distribution will be presented later. However, the seasonal variation is worthy of some comment:

(a) Maximum evaporation is usually recorded in January (December for a few stations).

(b) Lowest values are recorded in June and July.

(c) January totals are mainly at least five times the July totals.

(d) About three-quarters of the annual evaporation occurs during the seven months, October to March.

(e) The total during the four months with lowest evaporation, May to August, is usually less than 15% of the annual value.

Range and Variability of Tank Evaporation

The range and variability of tank evaporation are of some practical importance, as they give some indication of the magnitude of the error involved in using average values as estimates for individual months or years. As in rainfall studies, the range and variability relative to the average value are of most significance. We may define

$$\text{Extreme range } \% = \frac{\text{highest total recorded} - \text{lowest total recorded}}{\text{average value}} \times 100$$

Similarly, as for rainfall (see for example Seelye 1946), the mean variability may be defined as the average departure, irrespective of sign, of the (annual or monthly) totals from their mean expressed as a percentage of the latter.

Calculations have been made for the year and for January using in each case the following stations, selected mainly for length of record: Ruakura, Appleby, Lincoln, Rudstone, and Alexandra. At these stations:

(a) For annual evaporation the extreme range varied from 18% at Ruakura to 42% at Lincoln. The average over the five stations was 29%.

(b) For January evaporation the average of the extreme range over the five stations was 56%; and the average of the mean variability over the five stations was 12%.

It is also of some interest to note that at all of the five stations extreme range and mean variability were much less for evaporation (both monthly and annual) than for rainfall; on the average the evaporation range and variability were about one third of the rainfall range and variability respectively.

Association of Low Rainfall with High Evaporation

Rainfall and evaporation data for January for the same five stations show some association between low rainfall and high evaporation. The average evaporation for the four driest Januaries was about 10% greater than the average value for January over the whole period.

RELATION BETWEEN TANK AND OPEN-WATER EVAPORATION

The evaporation from a tank is greater than from a large expanse of water, such as a lake or reservoir, under the same meteorological conditions. In experiments carried out overseas, especially in the United States, reduction factors have been determined for several designs of evaporation tank, and the factors quoted in Table 1 are those judged most appropriate to the various types of tanks used in New Zealand with the water-level prevail-

ing at the time. Since then the water-levels have been raised in the main types of tank to bring them into line with those specified in U.S.A. The revised reduction factors are:

New Zealand sunken pan 0·79.
New Zealand raised pan 0·69.

For experimental determination of reduction factors reference should be made to Harding (1942), Young (1947), and especially Kohler (1952). For the effect of changes in water-level see Bonython (1950) and Ventikeshwaran, Jagannathan and Ramakishran (1959).

The variations in reduction factor can be summarised as:

(1) Reduction factors are larger for sunken pans than for raised pans.

(2) The water depth in the tank is not critical, so long as it is more than 2 ft.

(3) The factor increases to nearly unity for a sunken pan with diameter at least 12 ft. In a raised pan the factor continues to increase up to a diameter of about 20 ft.

(4) There is a complicated seasonal variation in the reduction factor with respect to a lake, on account of heat storage effects.

(5) The colour of the tank has an effect. The duller and darker the tank, the lower the reduction factor.

INDIRECT ESTIMATION OF TANK EVAPORATION

In the absence of actual measurements indirect methods may be used to estimate the equivalent tank evaporation from available weather data. The method using the formula derived by Penman is outlined below.

Penman's Equation

The equation derived by Penman (1948) was:

$$E_o = \frac{\Delta H + \gamma E_a}{\Delta + \gamma} \qquad (1)$$

where E_o is the evaporation in mm from the English tank (6 ft sunken pan)

H is the net amount of radiant energy available expressed in the equivalent of mm of evaporation;

Δ is the slope of the curve of saturation vapour pressure against temperature, in mm per °F;

γ is the constant of the wet-bulb equation (0·27 in these units);

and E_a is a linear function of wind-speed and saturation deficit.

For satisfactory testing of Penman's equation it is necessary to have measurements of incoming solar radiation and back radiation, besides the usual observations of tank evaporation, water temperature, air temperature,

humidity, and wind-speed. Penman showed that agreement between calculated and observed evaporation was satisfactory for the 6 ft English tank.

The function E_a may be obtained directly from measurements of air temperature, water temperature, wind speed, and evaporation. Penman showed that for the English tank

$$E_a = 0{\cdot}35\ (e_a - e_d)\ (1 + u_2/100)$$

where $e_a - e_d$ is saturation deficit in mm.

and u_2 is wind speed in miles per day at a height of 2 metres above ground.

Effect of Size and Type of Tank

Equation (1) was determined for the standard English evaporimeter – a 6 ft square sunken pan. Some adjustment is needed if it is to be applied to the smaller New Zealand sunken pan and also to the New Zealand raised pan.

Kohler (1952) suggested that the so-called constant "γ" cannot be expected to have the same value for different tanks. Using the Lake Hefner observational data, he obtained satisfactory agreement by multiplying γ by an empirical factor, which was different for the two tanks considered. For convenience this empirical factor will be denoted by "k". The values of k obtained by Kohler were:

Raised pan 2·5.
Sunken pan, circular, 6-ft diameter (Bureau of Plant Industries, "BPI", type) 1·5.

Kohler also showed that the form of E_a varied with the size and type of tank. He did not attempt to account for the discrepancy between his results and those of Penman for similar though not quite identical 6-ft sunken pans. He gave the reduction factor of the BPI pan as 0·94.

Some of the discrepancy may be due to the difference in the shape and area of the two pans. However, it is probable that most of the discrepancy is due to the somewhat different methods adopted by Kohler for measuring wind-speed and saturation deficit. The value of k obtained is in fact fairly sensitive to small variations in these measurements.

Testing Penman's Equation for New Zealand Sunken Pan Evaporimeter

In testing Penman's Equation for New Zealand conditions, the following procedure was adopted:

(a) For most stations, averages of monthly tank evaporation over a period of years were tested, and winter values were not included.

(b) The incoming radiation (the main term contributing to H) was estimated by the method of Gabites (1951). Briefly, this consists of multiplying Gabites' estimates for clear skies by $(0{\cdot}33 + 0{\cdot}67n/N)$,
where n is the actual sunshine and N the possible sunshine.

(c) The back radiation R_B (another term affecting H) was estimated by the method recommended by Penman, namely:

$$R_B = \sigma T_a^4 (0{\cdot}56 - 0{\cdot}92\, e_d) (0{\cdot}10 + 0{\cdot}90n/N)$$

where σ is Stefan's constant,

T_a is temperature in degrees Absolute,

and e_d is vapour pressure in mm.

(the 9 a.m. value of e_d was used as an approximation to the mean of 24 hr).

(d) The original form of E_a as proposed by Penman was used, but since New Zealand cup anemometers are mainly at a height of 20 ft above ground level, the equation was modified to

$$E = 0{\cdot}35 (1 + 0{\cdot}85 \times 10^{-2} u_6) (e_a - e_d)$$

where u_6 is mean wind speed in miles per day at a height 6 metres above ground level.

The saturation deficit $e_a - e_d$ was obtained by an approximate method, by subtracting the mean 9 a.m. vapour pressure in mm from the saturated vapour pressure corresponding to the approximate mean temperature.

Results of Testing

Using γ uncorrected (0·27), the calculated values of tank evaporation were much too high; thus in January they averaged about 25% higher than the observed values. By a process of trial and error it was found that best agreement was obtained by using a value of 0·6, that is, a value of k of 2·2. Table 2 below summarises the results of the tests.

TABLE 2—Summary of Results for Tests Made Using K = 0·6.

Month of Year	Evaporations Tested	Maximum Difference calc.-obs. (in.)	Station or Year	Root-mean-square Differences calc-obs. (in)	Root-mean-square Differences calc-obs. % of Observed Value
(i) January	Avge. values for 11 stations	−0·8	Lake Grassmere	0·41	7
(ii) April	Avge. values for 12 stations	−0·6	Lincoln Winchmore	0·37	14
(iii) November	Avge. values for 10 stations	−0·8 +0·8	Lake Grassmere Wellington	0·52	12
(iv) January	Individual adjusted totals for Wellington (1950–54)	+0·5	1952	0·27	5
(v) June	Individual adjusted totals for Wellington (1949–54)	+0·2	1954	0·13	11
(vi) October	Individual adjusted totals for Wellington (1949–53)	+0·3	1953	0·14	5

NOTE: Adjustment of Wellington totals was carried out by means of a Piche atmometer, correcting evaporations on days of considerable rain and on a few other days when they appeared to need correction. See "Analysis of Errors and Their Effect", Appendix.

The correlation coefficient between the calculated and observed values in January, April, and November (corresponding to (i), (ii) and (iii) in Table 2) was 0·97. It will be seen that the root-mean-square differences in (i), (ii) and (iii) do not vary greatly with the month of the year, but the relative differences (last column) are least in summer and greatest in the cooler months. Considering the errors and approximations involved, the agreement is reasonably satisfactory, especially in the warmer months.

ESTIMATION OF OPEN-WATER VALUES

The modified Penman equation was used to calculate the equivalent of sunken-pan evaporation for other stations, and from these estimates the open water evaporations were obtained by applying a factor of 0·87. The number of climatological stations which include measurements of both wind-speed and sunshine is rather limited, and many of the stations used were aerodromes with Dines pressure-tube anemometers at a height of 33 ft above the ground. For these stations a correction factor of 0·91 was applied to the wind-speeds to reduce them to the equivalent value at a height of 20 ft.

Open-water evaporations were calculated from meteorological data for the following stations: Napier, Wanganui, Masterton, Hanmer, Hermitage (Mt Cook), Adair (Timaru), Gore; and the aerodromes at Kaitaia, Tauranga, Rotorua, Gisborne, New Plymouth, Hokitika, and Taieri.

The following additional records were made available from evaporimeters owned by other organisations:

Wither Hills, Blenheim (Agriculture Dept.); raised pan.

Harper River, Lake Coleridge (Electricity Dept.); sunken pan, Electricity Dept. type.

Lake Mahinerangi, Waipori Falls, Otago (Dunedin City Corporation); sunken pan.

These tank evaporations were converted to the equivalent open water evaporations by the use of the appropriate reduction factors.

All estimates of open water evaporation obtained by these various means were plotted in Fig. 3.

DISCUSSION OF RESULTS

Importance of Exposure

The importance of the exposure of the station, especially with respect to wind, is shown by the differences in estimated open water evaporation between the following pairs of stations:

(1) Ruakura, slightly sheltered but typical of the Waikato countryside; annual open-water evaporation 28 in.; average wind-run 110 miles per day.

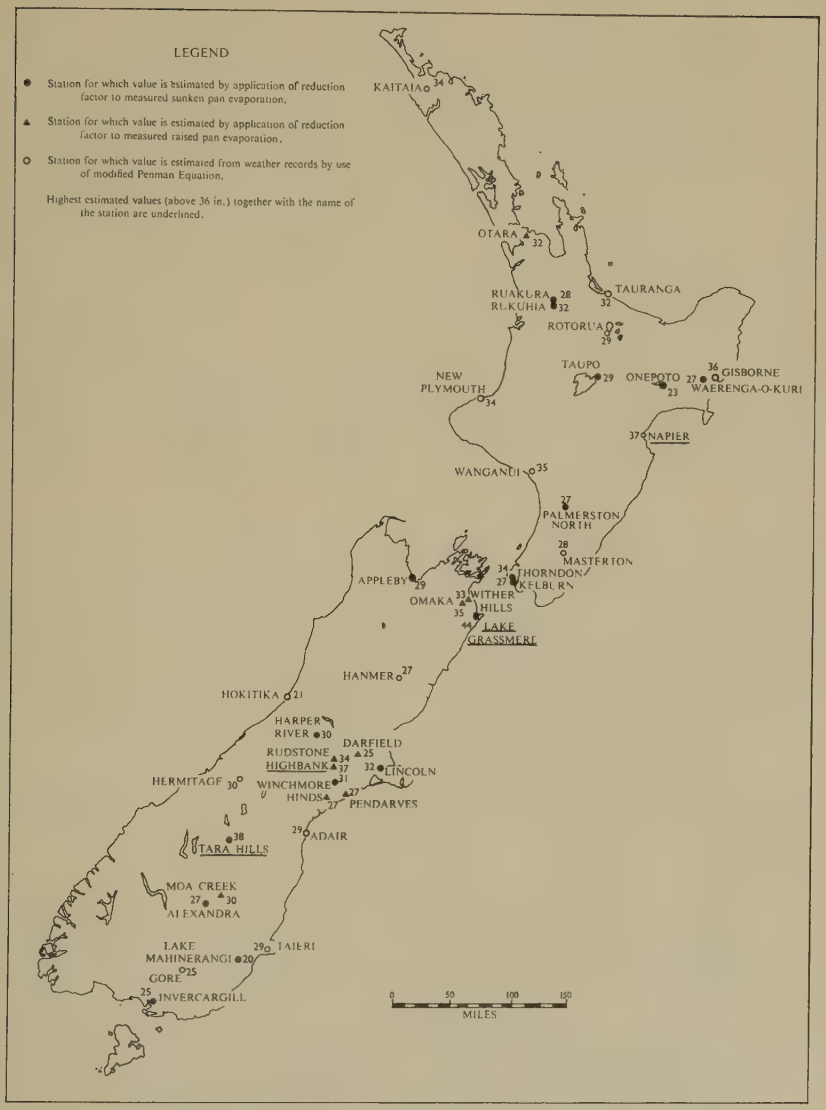

FIG. 3—Estimated annual open water evaporation in New Zealand (inches).

Rukuhia, 5 miles to the south of Ruakura, on top of a hillock, more exposed to wind than Ruakura; annual open-water evaporation 32 in.; average wind-run 170 miles per day.

(2) Kelburn, somewhat sheltered for Wellington; annual open-water evaporation 27 in.; average wind-run 210 miles per day. Thorndon, 1 mile to the north-east of Kelburn, a good average exposure

for Wellington; annual open-water evaporation 34 in.; average wind-run 260 miles per day. The Thorndon site was also a little warmer than Kelburn.

(3) "Rudstone", Methven, somewhat sheltered; annual open-water evaporation 34 in.; average wind-run 120 miles per day. Highbank Power Station, Methven, 4 miles south-east of "Rudstone", close to the Rakaia Gorge, which is very exposed to north-westerly winds; annual open-water evaporation 37 in.; average wind-run 190 miles per day.

Except in the case of Wellington, the two stations of each pair have had exactly the same type of evaporimeter.

Any generalisations regarding evaporation as shown in Fig. 3 must take some account of varying exposures. In particular, Darfield, Pendarves, and Hinds in Canterbury are all rather sheltered. On the other hand, aerodromes are often somewhat over-exposed in comparison with the surrounding countryside.

The Pattern of Evaporation in New Zealand

The evaporations plotted in Fig. 3 are not homogeneous, being based partly on evaporation data from four different types of tanks and partly on estimated values. Nevertheless certain features do stand out:

(1) The highest value shown, 44 in., at Lake Grassmere, is associated with the high wind speeds of Cook Strait combined with a sunny climate.

(2) In the South Island the next highest values of 35 to 38 in. appear in parts of the high country and upper plains of Canterbury and North Otago and in the Wairau Valley of Marlborough.

It is noteworthy that even the higher rainfall areas of Canterbury in and close to the Alps (such as the Hermitage and Harper River) have evaporation about as high as the lower plains and the coast.

(3) In the North Island the range of values is lower. Highest evaporations shown of 34–37 in. occur in coastal areas. The east coast has greater evaporation than the cloudier and more humid areas of Taranaki and Northland, but the difference is quite small.

(4) The lowest values shown are at Lake Mahinerangi, Hokitika, and Onepoto (Lake Waikaremoana). At the two last-mentioned places the low evaporations are associated with humid conditions, but Hokitika also has comparatively low wind-speeds.

(5) For a large proportion of the stations shown (35 out of 41) open-water evaporation lies between 24 and 36 in., that is, within 20% of 30 in.

TANK EVAPORATION AND EVAPORATION FROM PASTURES

Tank evaporimeters are designed primarily to give a measure of open-water evaporation. Nevertheless, in the search for some indication of the evaporation and transpiration occurring from crops and pastures, and lacking any better method, a number of agricultural stations have installed tank evaporimeters.

The only form of evaporation from pastures that can be directly related to current meteorological conditions is the water loss from adequately watered land, sometimes known as the "water need" or "potential evapotranspiration" (p.e.). Penman (1948) showed that in south-east England the p.e. could be satisfactorily calculated from the open-water evaporation by the use of the following factors.

May–August	0·8
March, April, September, October	0·7
November–February	0·6
Year	0·75

If these factors are correct for New Zealand conditions, then from the reduction factors previously given, it should be possible to calculate the following factors to be applied to tank evaporation to obtain p.e.:

TABLE 3—Estimated Values of Factors to be Applied to New Zealand Tank Evaporation to Obtain p.e.

(a. With present water-level. b. With old water-level.)

Months	3 ft Sunken Pan		Raised Pan	
	a	b	a	b
Nov.–Feb.	0·63(0·65)	0·70(0·7)	0·55(0·55)	0·58(0·6)
Mar., Apr.				
Sept., Oct.	0·55(0·55)	0·61(0·6)	0·48(0·5)	0·50(0·5)
May–Aug.	0·47(0·5)	0·52(0·5)	0·41(0·4)	0·43(0·45)
Year	0·59(0·6)	0·65(0·65)	0·52(0·5)	0·54(0·55)

NOTE: The results of the calculations are given initially to two decimal places, but in view of the comparatively low degree of accuracy claimed by Penman they should be "rounded off", as shown, to the nearest ·05.

The only extensive series of New Zealand observations known to the author from which the p.e. may be obtained directly are those of Rickard (1957) at Winchmore Irrigation Research Station, on the Canterbury Plains. From the measured moisture deficit (with irrigation treatment at 20% soil moisture) for 6 periods from December to February 1955–56 (quoted) and 5 periods from November to February 1956–57 (the latter given in a private communication) the average ratio of sunken pan to p.e. was 0·61, varying from 0·54 to 0·74. In other words, the "summer" value of the

factor on the Canterbury Plains is of the same order as that calculated from Penman's results in south-east England. On the other hand, Rickard's measurements appear to indicate that at Winchmore the value of the factor is just as high in September and October as from November to February.

It would be unwise to assume that the factor has exactly the same value in other parts of New Zealand as on the Canterbury Plains. In particular, it may well be appreciably different in more humid areas such as the Waikato.

Rickard's work was primarily concerned with testing the Thornwaite (1948) method of calculating potential evapotranspiration. For further details of the application of the Thornthwaite method in New Zealand see Gabites (1956).

CONCLUSIONS

(1) The most serious errors in tank evaporation occur on days of considerable rainfall, especially when accompanied by high winds. In winter, individual daily totals are frequently quite unreliable.

(2) The relative range of monthly and annual tank (and open water) evaporation from year to year are only about a third of the relative range and variability of monthly and annual rainfall respectively.

(3) A modification of Penman's equation for estimating monthly tank evaporation from weather records gives satisfactory agreement with observed monthly tank evaporation in New Zealand.

(4) From measurements made overseas it is believed that the open water evaporation may be obtained by applying a factor of 0·79 to the evaporation from the New Zealand sunken pan or 0·69 for the New Zealand raised pan.

(5) In summer the water-need of pasture may be calculated approximately from the New Zealand sunken pan evaporation by multiplying by a factor of 0·65 or from the New Zealand raised pan by multiplying by a factor of 0·55.

ACKNOWLEDGMENTS

Thanks are expressed to the Ministry of Works for supplying records from the raised pan evaporimeters at Omaka, Pendarves, and Hinds; and also to the Agriculture Department, Electricity Department, and Dunedin City Corporation for supplying records.

The writer wishes to thank Mr N. G. Robertson and Dr J. F. Gabites for their constructive criticism of the manuscript.

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APPENDIX

Analysis of Errors in Tank Evaporation at Wellington

In view of the frequent association of rain and strong winds at Wellington, it might be expected that errors due to rain would occur rather frequently at this station. An examination of Wellington's individual daily tank readings has been made in conjunction with simultaneous readings from a Piche atmometer. This instrument operates inside a Stevenson screen and is not affected by rain. It may be used to provide a rough estimate of the true tank evaporation. It appears that at Wellington on rainy days errors of type (1) above (i.e., calculated evaporation too large, due to splash-out) predominate, probably combined at times with loss from wind. There have been winter days at Wellington when the net loss of water from the tank has been more than half an inch, although the estimated true evaporation would amount to only a twentieth of an inch!

Errors of types (1) and also (ii) (i.e. calculated evaporation too low, due to more efficient catch) affect readings at all stations, but more especially those with high rainfall, such as Onepoto (Lake Waikaremoana). In the routine checking at Wellington of observations for all stations, the usual procedure has been to reject any calculated evaporation which is excessively high or negative and simply replace it by "nil". This procedure is obviously rough – some incorrect values are untouched, while on most days for which

"nil" is written appreciable evaporation does occur. Nevertheless, examination of the Wellington data indicated that totals were not seriously affected in most months. In particular:

(a) The maximum differences in individual monthly totals from those deduced with the aid of the Piche atmometer amounted to only 0·3 in. in summer, but were as high as 0·6 in. in other seasons.

(b) The differences in average monthly totals over a period of 7–8 years were negligible in summer and amounted to 0·1–0·2 in. in other seasons.

(c) There was no evidence of "bias" in the average monthly totals, except in winter, when the totals were nearly always somewhat too high.

In general, it seems likely that at most other stations individual and average monthly totals are somewhat less affected by errors due to rain than at Wellington. However, at nearly all stations monthly totals during winter are only very approximate. The evaporation during these months is, however, so small that annual totals are very little affected.

STUDIES IN RADIOACTIVE FALLOUT IN NEW ZEALAND

Part 1—THE MEASUREMENT OF RADIO-CAESIUM, -STRONTIUM, -BARIUM, AND -CERIUM IN RAINWATER

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(Received for publication, 18 May 1961)

Summary

An analysis scheme which has been used during the past year to determine ^{137}Cs, ^{144}Ce, ^{90}Sr and ^{140}Ba in every rain at Lower Hutt, New Zealand, is presented. Rainwater is collected in a polythene tank containing carriers, and soluble kationic material is absorbed by a Dowex 50 resin column. The above isotopes are separated by elution with 0·7 M HCl, 1·5 M ammonium lactate and 4 M HCl. The caesium and cerium fractions are radiochemically purified before counting, the former with a gamma spectrometer and the latter with a low-background beta counter. The ^{90}Sr and ^{140}Ba are reabsorbed on ion exchange columns and their ^{90}Yt and ^{140}La daughters are eluted with pH 3·8 ammonium citrate, precipitated with yttrium oxalate and counted in a low background counter. The recovery of each carrier is determined chemically. Inherent errors in the method due to counting statistics and decay of activity are equivalent to deposited activities of about 5, 0·2, 0·4, and 0·8 micro curies/sq. mile for ^{137}Cs, ^{144}Ce, ^{90}Sr, ^{140}Ba respectively, at the 90% confidence level.

Introduction

As part of a programme of radioactive fallout measurements it was decided to measure routinely in rainfall the three isotopes ^{137}Cs, ^{90}Sr, and ^{140}Ba. When the scheme had been in operation for about six monhs ^{144}Ce was also incorporated. Simplified decay schemes of the isotopes are taken from Strominger, Hollander and Seaborg (1958).

^{137}Cs —27 yr→ ^{137}Ba

92% 0·52 MeV β
0·66 MeV γ
8% 1·2 MeV β

^{90}Sr —28 yr→ ^{90}Yt —64 hr→ ^{90}Zr

0·61 MeV β | 2·2 MeV β

^{140}Ba —12·8 dy→ ^{140}La —40 hr→ ^{140}Ce

60% 1·0 MeV β | up to 2·2 MeV β
0·48 MeV β | Mainly 1·6 MeV γ
up to 0·54 MeV γ

^{144}Ce —285 dy→ ^{144}Pr —17·3 min.→ ^{144}Nd

75% 0·31 MeV β | 98% 3·0 MeV β
0·18 MeV β
up to 0·14 MeV γ | about 2% γ

It was decided to estimate ^{137}Cs by using a single channel gamma scintillation spectrometer and the ^{90}Sr and ^{140}Ba by counting their short-lived daughters ^{90}Yt and ^{140}La in a low background beta counter. ^{144}Ce was also best estimated in the low background beta counter. It was considered that the loss of sensitivity involved in counting the daughters ^{90}Yt and ^{140}La rather than the equilibrium mixtures $^{90}Sr/^{90}Yt$, $^{140}Ba/^{140}La$ was more than offset by the possibility of checking the radio-purity of the samples by measuring daughter half-lives.

There have been a large number of papers in recent years concerned with the measurement of fission products in natural waters, irradiated fuels, radioactive effluents, soils, and biological materials. It is considerably easier to measure radioisotopes in rainwater than in these other matrices. This is firstly, because the isotopes are generally water soluble and more easily available; secondly, because there is less interference with the chemical separations by the solid material in the matrix; and thirdly, because carrier recoveries and analytical losses are more readily found, as the natural occurrence of stable isotopes of the radioelements being determined is negligibly small. It was considered that ion exchange techniques would provide a more satisfactory method for the initial concentration and separation of fission product activities than the usual evaporation and precipitation techniques. After this initial separation the radioelement in each fraction is radiochemically purified, the activity is found, and finally, processing losses are determined by measuring the recovery of initially added inactive carrier. The procedure outlined in this paper combines features from several published papers into a scheme which has proved reliable for routine operation. These papers include those by Martell (1956), Milton and Grummitt (1957), Stanley and Kruger (1956), Langford (1957), and Osmond *et al.* (1959).

EXPERIMENTAL

Collection of Sample

Rainwater is collected in a rectangular wooden tank approximately 40 in. × 36 in. × 6 in. deep, lined with 5 mm thick polythene sheet and with a drainhole in one corner. The tank is supported on a wooden frame about 42 in. high situated in the open, clear of buildings. The rainfall is measured by a standard 5 in. rain gauge placed nearby. At the start of a collection, the collector is filled to about ½ in. deep with distilled water containing 40 mg each of Sr^{++}, Ba^{++}, and Cs^{+}, and 5 mg of Ce^{IV} as nitrates. The distilled water level is kept up during dry spells. After each rain the collector is scrubbed out with a rubber paddle into polythene buckets and washed out with distilled water. The water is then stored in the head tank of the ion exchange system (see Fig. 1) for a day before use, to allow most of the sediment to settle to the bottom.

Ion Exchange Equipment

The equipment used for the ion exchange separations is shown in Fig. 1. The head vessel is a cylindrical drum about 15 in. diameter and 18 in. deep lined with polythene sheet and capable of holding about 8 gallons of

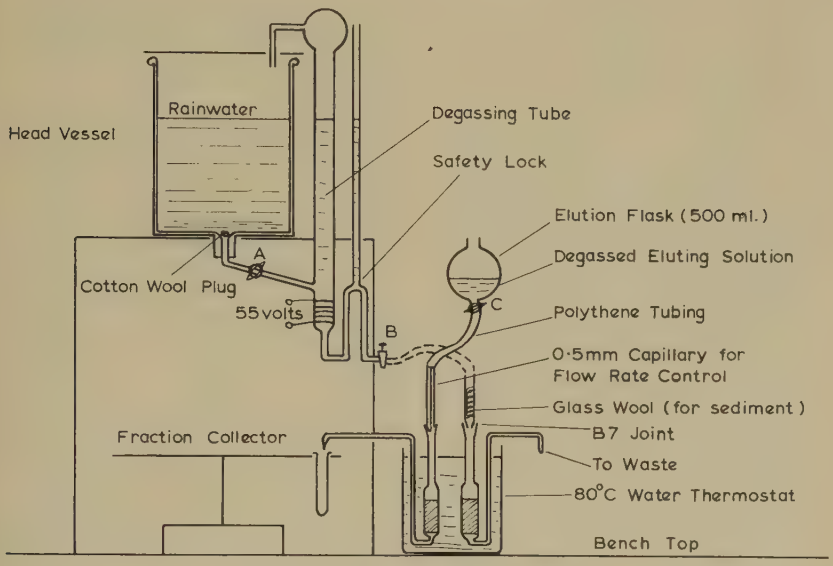

Fig. 1—Ion exchange equipment.

water. Since the ion exchange operations are carried out at 80°C it is necessary to deaerate the rainwater to prevent bubble formation in the resin. The water is deaerated by boiling in the 1 in. diameter tube constructed with the safety loop shown in the diagram to prevent damage to the heater if the head vessel runs dry. The heating coil is Nichrome strip, 74 cm of 0·025 in. by 0·003 in. thick (22 Ω/yd), and the power is supplied from a 55 V tapping from a 230 V transformer. The thermostat bath is a cylindrical Pyrex jar 14 in. deep and 12 in. diameter. The water bath is controlled at 80° ± 3°C by a Sunvic Bimetallic Stem Thermostat.

The construction of the large resin columns used for the initial absorption and separation of caesium, cerium, strontium, and barium, and the small resin columns used for milking ^{90}Yt from the strontium, and the ^{140}La from the barium is shown in Fig. 2. The resin used in both types of column is Dowex 50 — X8, mesh 100–200 supplied in the hydrogen form. A batch of resin is eluted with 6N HCl until no further coloured impurities appear in the effluent, then washed with distilled water and the lighter particles discarded after the heavier ones have settled out from distilled water in a measuring cylinder. The larger ion exchange columns are filled with 21 ml of settled resin, and the smaller columns filled to a height of 6 cm. After converting the columns to the ammonium form by eluting with 400 ml of 1·5 M ammonium lactate pH 7·0 and washing with distilled water, the resin is resuspended by backwashing with distilled water while in the water bath and allowed to settle under gravity. The columns are then ready for use.

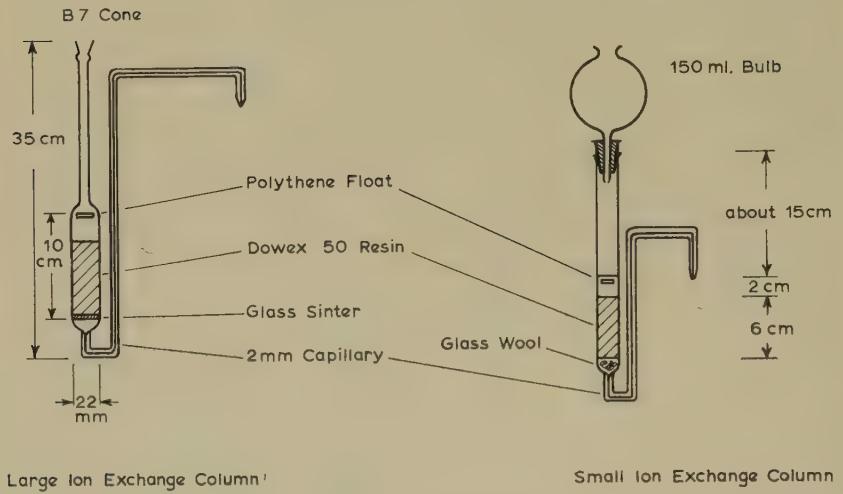

FIG. 2—Ion exchange columns.

The Initial Isotope Separations

The flow sheet for the determination of ^{137}Cs, ^{144}Ce, ^{90}Sr and ^{140}Ba is shown in Fig. 3. The rainwater is firstly allowed to flow via a glass wool filter plug, through a large resin column at about 15 ml/min to absorb

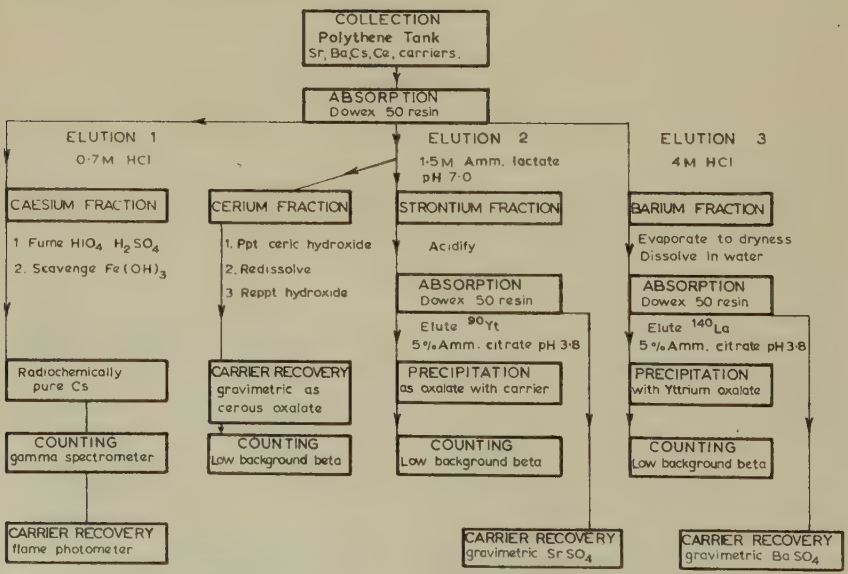

FIG. 3—Flowsheet – The Determination of ^{137}Cs, ^{90}Sr, ^{140}Ba and ^{144}Ce in rainwater.

the carriers and their associated fission product activities. The subsequent elution schedule to separate the four isotopes into different fractions is given in Fig. 4. Each eluting solution is put in 500 ml flasks like the one shown

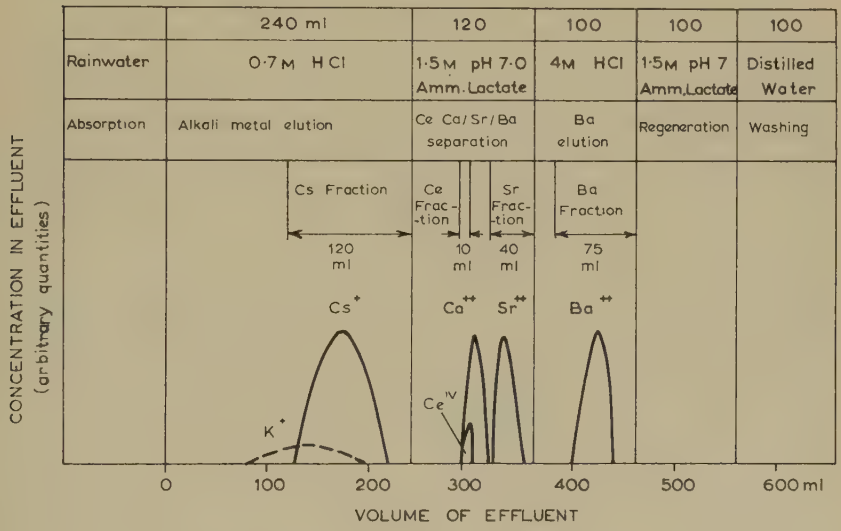

FIG. 4—Ion exchange separations for ^{144}Ce, ^{137}Cs, ^{90}Sr and ^{140}Ba in rainwater.

in Fig. 1. The flasks are stoppered and evacuated for a few minutes with a water pump through Tap C. The tap is closed, the flask shaken vigorously and then re-evacuated. This procedure is repeated several times to degas the eluting solutions. The eluting solutions are passed through 0·5 mm diameter capillary tubing the length of which is adjusted to about 10 cm to set the flow rate at about 1 ml per minute.

In the first elution with 0·7 M HCl the effluent is collected in a 250 ml measuring cylinder. The first 120 ml containing much of the sodium and potassium deposited in sea spray and dust is discarded and the remaining 120 ml is collected in a 250 ml beaker and kept as the *caesium fraction*.

The second elution is with 120 ml 1·5 M ammonium lactate pH 7·0 and the effluent is collected in 4 in. × ½ in. test tubes on a fraction collector, set at 4 min intervals. The position of the cerium peak is apparent from its yellow colour in the 50–60 ml interval. These tubes are kept as the *cerium fraction*. The tubes that follow after cerium are each tested for calcium (deposited in dust in the rainwater collector) by addition of a few drops of saturated ammonium oxalate solution until a negative test is obtained. Calcium usually appears in the 50–80 ml interval. The remaining effluent containing strontium, is collected in a 100 ml measuring cylinder as the *strontium fraction*.

The third elution is with 100 ml of degassed 4 M HCl. The first 25 ml is discarded and the remainder collected in a 250 ml beaker as the *barium*

fraction. The column is then prepared for the next sample by eluting successively with 100 ml of degassed 1·5 M ammonium lactate pH 7, followed by 100 ml of distilled water.

Radiochemical Purification and Counting of the Separated Isotopes

Caesium Fraction

The caesium fraction in the beaker is evaporated to dryness on a steam bath and radiochemically purified by a process described by Langford (1957). Possible radio-ruthenium contamination is first removed by adding 20 ml water, 1·2 g periodic acid and 4 ml conc. H_2SO_4 to the residue in the beaker and boiling down on a hot plate until only 2 ml of H_2SO_4 remain. After cooling 15 ml water is added and boiled down to 1 ml. After cooling, 15 ml water is again added and boiled until only a few drops of H_2SO_4 remain. The residue is finally purified by two ferric hydroxide scavenging steps. After washing into a 6 in. × 1 in. centrifuge tube, 0·5 ml $FeCl_3$ (50 g Fe/litre) and a slight excess of 50% NaOH are added and the tube filled two-thirds full with water. The tube is centrifuged and the supernatant liquid decanted into the original beaker. The $Fe(OH)_3$ precipitate is washed with 5 ml water, centrifuged and the supernatant liquid again added to the beaker. The ferric hydroxide scavenging is repeated and the combined caesium fraction and washings evaporated to dryness, the residue transferred to a clean 4 in. × $\frac{5}{8}$ in. plastic test tube, and the beaker washed out with sufficient water to make the total volume in the tube up to 5 ml. The ^{137}Cs activity is counted as described later. Finally the caesium fraction in the plastic test tube is poured into a 100 ml graduated flask, washed out with water and made up to 100 ml. The carrier recovery is determined by flame photometry.

Cerium Fraction

The fraction collector tubes containing the cerium fraction are washed into a 250 ml beaker, 1 ml 30% H_2O_2 added, and ceric hydroxide precipitated by ammonia. This step separates the cerium from the calcium which is also present in this fraction. The precipitate is dissolved in dilute HNO_3, and ceric hydroxide reprecipitated as before, filtered and washed with water. After redissolving in the minimum of 2N HNO_3 in the original beaker, about 15 ml of distilled water is added followed by about 7 ml of saturated oxalic acid. Ammonia is added dropwise until precipitation of cerous oxalate starts, then the solution is stirred vigorously for at least one minute. After standing for a few hours the precipitate is mounted on a filter paper disc and counted as described in the section on sample preparation.

Strontium Fraction

The strontium fraction is acidified to pH2 or less with about 25 ml 2N HNO_3 and the solution passed through a small ion exchange column to reabsorb the strontium. The column is immediately eluted with 30 ml

pH 3·8 ammonium citrate solution (5% with respect to citrate) to remove ^{90}Yt and give a start time for the build up of ^{90}Yt. After leaving the column for partial or complete establishment of radiochemical equilibrium, the ^{90}Yt is eluted into a 150 ml beaker containing yttrium carrier solution (4·00 mg Yt^{3+} as nitrate in 10 ml), by passing 30 ml pH 3·8 ammonium citrate solution through the resin column. The volume of solution in the beaker is thus about 40 ml and to this is added 15 ml of saturated oxalic acid solution, and the mixture stirred briskly for at least 1 min, otherwise precipitation of yttrium oxalate may be incomplete. The precipitate is allowed to settle for several hours, and then filtered using a Tracerlab E-8B precipitation apparatus on to a $^{13}/_{16}$ in. diameter circle of No. 42 Whatman filter paper which has been weighed and counted previously as described under "Preparation of Samples for Counting". The sample is re-weighed to give (y) the yttrium carrier recovery fraction, then placed on the same stainless steel disc and covered with the same cellotape cover as before and the activity counted overnight. When it is clear that the ^{90}Yt activity measurement is of the right order, and the column will not have to be re-milked, the strontium is eluted from the small column with 90 ml of 1·5 M ammonium lactate. The strontium carrier recovery fraction (Y) is found gravimetrically by precipitation of $SrSO_4$ in 50% alcohol-water mixture, and the column is eluted with 100 ml water before re-use.

Barium Fraction

The barium fraction in the beaker is evaporated to dryness on a steam bath, the residue dissolved in 100 ml water and the barium reabsorbed on a small ion exchange column. The column is eluted with 30 ml pH 3·8 ammonium citrate solution to remove ^{140}La and to give a start time for the subsequent build-up of the ^{140}La activity, which will be a maximum after 5 days. The ^{140}La is eluted into a beaker containing yttrium carrier, precipitated and counted exactly as described for ^{90}Yt. The barium is then eluted with 90 ml 1·5 M ammonium lactate as before, the volume made up to 200 ml with water and $BaSO_4$ precipitated from the boiling solution by adding 50% H_2SO_4 dropwise. The barium carrier recovery factor (Y) is calculated from the weight of $BaSO_4$.

Counting Equipment and Sample Preparation

Gamma counting of ^{137}Cs is carried out using a Baird Atomics single channel scintillation spectrometer, Model 8200 with a 2 in. thallium activated sodium iodide, well-type crystal detector, Model 810. The spectrometer is adjusted to count the 0·662 MeV peak of ^{137}Cs between the approximate limits of 0·64 to 0·68 MeV. An Amersham CDR 4 reference solution of ^{137}Cs calibrated to $\pm$ 2% against the Radiochemical Centre Standard is used as the ^{137}Cs activity standard. Under normal operation the background of the apparatus is 9·5 $\pm$ 0·1 c.p.m. and the geometry factor for ^{137}Cs is about 0·060.

Beta counting of ^{90}Yt, ^{140}La and ^{144}Ce/^{144}Pr is carried out using a Tracerlab CE-14 Low Background Beta Counter. This equipment is used with

Tracerlab G-1 Geiger gas and one CE-14P-2S and one CE-2P-2L central flow counter which have 1·3 in. and 2·0 in. diameter windows of 0·9 mg/cm² aluminised Mylar film. Prior to the counting of a sample of any of these activities a blank is counted. This consists of a $^{13}/_{16}$ in. No. 42 Whatmans filter paper disc placed in a depression stamped in a 0·20 in. thick 1 in. diameter stainless steel planchette and lightly covered with a disc cut from cellotape. For each of the above fission products the activity absorbed on a precipitate is filtered on to the weighed filter paper disc previously counted in the blank count. The filtration is carried out using a Tracerlab E-8B precipitation apparatus. After washing with water, drying with acetone, and weighing the filter disc to find the carrier recovery, it is put back on to the original stainless steel planchette and the cellotape cover pressed on firmly. Reagent blanks are determined periodically and a correction made where necessary. Calibration curves showing the effect of sample weight on measured activity have been determined and corrections made if necessary when recoveries are low. Blank runs containing only carrier solutions are periodically done to check for build up of activity in collector or ion exchange columns.

The standards used for this work are:

Amersham Code SIR 4 Standardised $^{90}Sr/^{90}Yt$ Reference Solution standardised to $\pm$ 2%.

Amersham 1 mc solution $^{144}Ce/^{144}Pr$ calibrated by 4π beta proportional counting with a standard deviation of less than 5%.

The ^{90}Sr standard was diluted in 0·1 N acid and aliquots absorbed on the small ion exchange columns. To check counter geometries ^{90}Yt is eluted and counted in the same way as described previously. The cerium standard was diluted in 0·2 N acid with the addition of carrier and aliquots precipitated and counted as described previously. The geometry factor for ^{140}La is assumed to be the same as for ^{90}Yt. Typical geometry factors (G) and background counts for the two flow counters and for 7 mg/cm² end window low background GM tubes, which were sometimes used in the anticoincidence shield are listed in Table 1:

TABLE 1—Typical Counter Characteristics

	Small Flow Counter	Large Flow Counter	GM4
Geometry Factor ^{90}Yt	0·47	0·52	0·19
Geometry Factor $^{144}Ce/^{144}Pr$	0·32	0·36	
Background (c.p.m.)	0·52	1·22	0·41

Calculation of Results

THE DEPOSITION OF ^{90}Sr AND ^{140}Ba

The deposition of ^{90}Sr and ^{140}Ba at the time of collection was calculated from:

$$D = \frac{kc}{G f_D f_b f_d Y y} \text{ micro-curies per sq. mile.}$$

where:

- D is the deposition of ^{90}Sr or ^{140}Ba in micro-curies per sq. mile.
- k is factor which equals 1·24 for 10·1 sq. ft. water collector.
- c is the average ^{90}Yt or ^{140}La activity in counts/min at mid-count.
- f_D is the ^{90}Sr or ^{140}Ba decay between sample collection and the start of the daughter build up.
- f_b is the daughter build up factor from the start of the build up until the elution of the ^{90}Yt or ^{140}La. This can be calculated from the equations for transient equilibrium given by Friedlander and Kennedy (1949).
- f_d is the daughter decay factor from the time of elution to the mid-counting time.
- Y is the carrier recovery factor for Sr or for Ba.
- y is the yttrium oxalate recovery factor.
- G is the counter geometry factor for ^{90}Yt or ^{140}La.

The Deposition of ^{144}Ce and ^{137}Cs

The deposition was calculated from the relationship:

$$D = \frac{kc}{G\ Y} \text{ micro-curies per sq. mile.}$$

where the symbols are analogous to those used previously.

Discussion

The above scheme has been used at the Nuclear Science Institute since about June 1959 to measure initially ^{90}Sr, ^{137}Cs and ^{140}Ba, and later ^{144}Ce in each rainfall. Approximately 80 rain samples were measured in the first year.

With care it was found that carrier recoveries of at least 90% would be obtained regularly for strontium, barium, and caesium. A complete analysis of a sample is generally obtained about 2 weeks after the end of the collection period, but this could be shortened considerably by allowing a shorter build up time for ^{90}Yt. Inherent errors in deposition at the 90% confidence level due to the statistics of counting and decay of activity are about 5, 0·2, 0·4, and 0·8 micro curies per sq. mile for ^{90}Sr ^{140}Ba, ^{137}Cs and ^{144}Ce respectively, for 1,000-minute counting times. Greater sensitivity might have been obtained by the beta counting of ^{137}Cs but this may have required more rigorous radio-purification and there was not sufficient counting time available on the low background counter. It has been found that in dry periods some of the barium carrier may be precipitated out with sulphate deposited in the collector if the water volume was not replenished with distilled water. Gravimetric perchlorate or dipicrylaminate determinations for caesium carrier were unsatisfactory and often 10% high

due to the presence of potassium deposited in the collector and not completely eliminated in the ion exchange separations. Determinations of caesium by flame photometry eliminated this difficulty. In early work in this project barium was eluted with 1·5 M ammonium lactate. It was found, however, that the eluted barium fraction after acidification was not completely retained on the small ion exchange columns as was the case with the strontium fraction. The present scheme of eluting the barium with 4M HCl with the necessary extra elution to return the resin to the ammonium form was considered more satisfactory than the alternative of precipitating the barium as carbonate from the lactate solution, filtering and redissolving before absorbing on the small column.

The ^{90}Sr content of the dirt-residue left in the head vessel after the processing of a sample has occasionally been determined and has always been found less than 5% of the activity of the sample. This could reasonably be due to ^{90}Sr in strontium carrier absorbed on the dirt, in which case the loss would be corrected for by the carrier recovery factor.

The determination of strontium and barium carrier recoveries and the possibility of detecting unwanted activities by measuring the half-lives of the ^{90}Yt and ^{140}La daughters makes this an inherently reliable method for ^{90}Sr and ^{140}Ba. The constancy of the $^{137}Cs/^{90}Sr$ ratio of 1·55 found in this laboratory, which agrees with that found by Osmond *et al.* (1959), and the thoroughness of Langford's radio-chemical purification method (1957) lend support to the reliability of the ^{137}Cs determinations.

The cerium activities measured in this work were found from 1 to 2 years after the last major nuclear weapons testing series (U.S.S.R., November 1958). It has therefore not been necessary to cut out 32 day ^{141}Ce by counting with a 110 mg absorber as recommended by Osmond *et al.* (1959). Since ^{90}Sr and ^{137}Cs have been isolated in other fractions the most likely radio-chemical impurities in the cerium are $^{95}Zr/^{95}Nb$, ^{106}Ru and ^{147}Pm. Other published sequential analyses remove Zr activities by precipitation of zirconium iodate from cerous solutions. After oxidation cerium activities are precipitated as the iodate, while yttrium acts as a hold-back carrier to keep ^{147}Pm in solution. In this work it was found that half-lives within 10% of the quoted ^{144}Ce figure were obtained when cerium hydroxide was precipitated from the cerium fraction, then redissolved and a cerous oxalate precipitate was counted for about 250 days. However, calculated activities could be four times too low due to chemical impurity of the cerous oxalate. A constant maximum specific activity could be achieved by purifying the cerium fraction in the manner described in this paper. As an additional check on the radio-chemical purity of the final cerium oxalate precipitate samples were also scavenged following the iodate methods described by Baus *et al.* (1957) and Osmond *et al.* (1959). The specific activity of the final purified cerium oxalate was less than 6% lower than that obtained without this additional treatment. Although more work should be done on fresh fission product mixtures or added radio-isotopes it seems likely that the method described here for fission product mixtures at least 1 to 2 years old will give ^{144}Ce activities within 10% of the true value without the ^{95}Zr and ^{147}Pm scavenging procedures described in earlier papers.

The present scheme has proved reliable in operation since June 1959 during which time, however, there has not been any large concentration of fresh fission products, to test the reliability of the radiochemical purification processes. Occasional measurement of the decay curve of ^{90}Yt has confirmed the radiochemical purity of these samples. The only positive indications of the presence of ^{140}Ba occurred shortly after the French Sahara tests, but the amount detected has been too small to enable this to be confirmed by determining the half-life of ^{140}La.

Acknowledgments

The authors would like to thank Mr T. A. Rafter for his suggestion and encouragement to start this project; Miss G. K. Oelsner who did much preliminary work; Mr K. Bargh for maintenance of the counting equipment, and the staff of Dominion Laboratory, Lower Hutt, for flame photometry analyses. The counting equipment used for this work was supplied by the United States Atomic Energy Commission under Contract AT (30–1) – 2167.

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TOPICAL APPLICATION OF INSECTICIDE SOLUTIONS TO MITES AND SMALL INSECTS

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Summary

The construction and procedure for use of an apparatus for topical application of insecticide solutions to mites and insects of a mass of approximately 20 μg are described. The apparatus consists of a self-filling micro-pipette mounted and arranged in the field of view of a stereoscopic microscope. The doses delivered to individual insects ranged from 0·0005 μl to 0·00005 μl according to the size of the micro-pipette used. Replicable results have been obtained using this method of treatment.

INTRODUCTION

Apparatus for topical application of insecticides to insects of the size of *Drosophila, Calandra, Musca,* etc., are known (Busvine, 1957, Hewlett and Lloyd, 1960), but for smaller organisms of a mass of approximately 20 μg such as mites and first instar larvae of Lepidoptera, no apparatus is available. Work on toxicity of insecticides to such organisms has therefore lacked the accuracy which can be obtained when known doses are applied to individuals.

An apparatus is described here whereby mites or insects, such as fruit-tree red spider mites and first instar larvae of leaf rollers and codling moths can be treated individually with doses of insecticides by topical application.

Basically the apparatus consists of a self-filling capillary tube of known volume. This tube is filled with the test solution and the contents forced on to the insect by compressed air. The whole procedure is carried out under a stereoscopic microscope.

APPARATUS (Figs 1 and 2)

Capillary Tube

The capillary tube is made by drawing out thick walled glass tubing. Suitable combinations of bore diameter and length to give desired volumes are: diameter 0·0166 mm; length 2·3 mm; volume 0·0005 μl: diameter 0·0058 mm; length 2·0 mm; volume 0·00005 μl.

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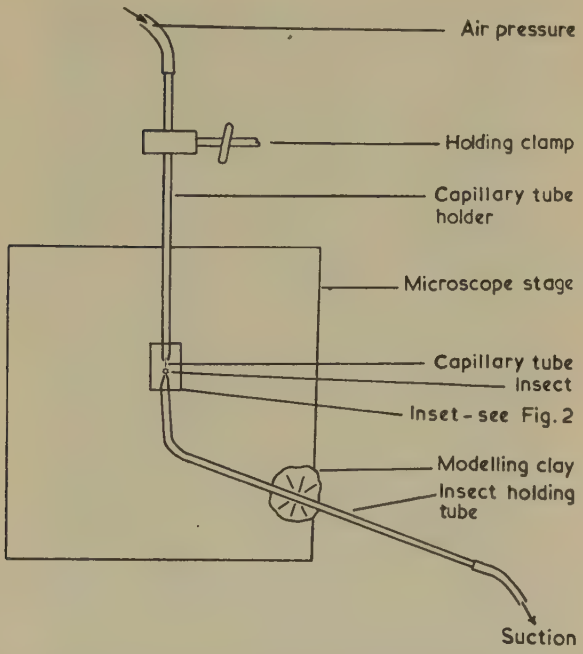


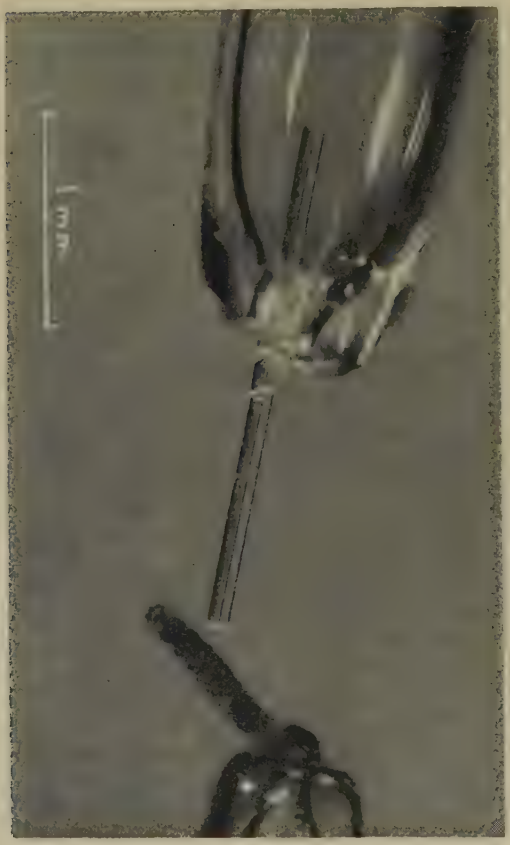
FIG. 1—Diagrammatic representation of the apparatus for topical application of insecticide solutions to small organisms.

Capillary Tube Holder

The capillary tube is held in the end of a 15 cm length of 3 mm diameter glass tubing. The holding end is drawn out and flamed to reduce its diameter to be just larger than the external diameter of the capillary tube it is to hold. The capillary is glued into position in its holding tube with cellulose acetate glue. The holder is rigidly fixed in a clamp and positioned so that the capillary tube is in the field of the microscope and about 3 cm above the microscope stage. The microscope is set up so that the stage is at approximately 30° to the horizontal.

Insecticide Solvent

Preliminary trials showed that kerosene is a suitable liquid for use in the apparatus. The viscosity of kerosene is low enough for easy expulsion from the tube and high enough for evaporation during the process of application to be neglected. It is also a suitable solvent for most organic insecticides and miticides and can be used with other solvents, e.g., xylol, when high concentrations of insecticides are required.



(*Photo—S. A. Rumsey*)

FIG. 2—Photo-micrograph of a 1st instar larva of a leaf roller being treated by the topical application apparatus.

At the volumes used for treatments, insects receive doses up to $^{1}/_{40}$th of their mass and with kerosene this rate has no apparent ill effect. These volumes are also sufficiently small to be placed discretely on any desired area of their body.

Calibration of Apparatus

The volume of each capillary tube is determined by measurement under a microscope and checked radiometrically. Repeated doses of kerosene solutions of ^{32}P — labelled tributyl phosphate were delivered on to small circles of absorbent paper. Volumes of liquid delivered were found to be within 5% of the estimated volumes.

Variation of Individual Doses

A check on the variation between individual doses from the same capillary tube was made radiometrically. Repeated single doses of ^{32}P — labelled tributyl phosphate in kerosene were applied to absorbent paper, adult female red spider mites, and newly hatched 1st instar leaf-roller larvae. The coefficient of variation for doses applied to paper was 5%, to mites 18%, and to larvae 5%.

Tests with radioactive solutions have shown that leaf-roller larvae receive a more complete and consistently even dose from the same capillary tube than do mites. This can be observed during tests and is probably caused by kerosene having a greater affinity for the integument of the leaf roller larvae than for that of the mite.

PROCEDURE FOR USE OF APPARATUS

Pretreatment Handling of Test Insects

Mites are shaken from their host plant on to sheets of white paper and a suction holder (see below) used to trap the required individual. It is possible to trap an individual mite in such a way that almost any desired surface will be exposed for treatment. In most tests carried out to date, it has been usual to trap mites so that the posterior region of the body is held on the suction holder and the antero-dorsal body surface is dosed. It is desirable to lightly anaesthetise 1st instar larvae of Lepidoptera with CO_2 before treatment. Such larvae are then trapped by the head capsule. Under the influence of the CO_2 they remain almost rigid and are dosed near the posterior end of the body. If they are not anaesthetised first, their wriggling movements make dosing difficult.

Filling of Capillary Tube

The kerosene solutions with which the tube is to be filled are placed in standard 2 ml hypodermic syringes. The tip of the needle of the syringe is touched to the exposed end of the capillary tube which fills itself. The syringe is steadied by pressing it on to a piece of modelling clay attached to the microscope stage. During the filling process some surplus liquid usually attaches itself to the outside surface of the capillary tube. This may be removed by wiping with a small piece of soft absorbent tissue paper held in forceps.

By using a hypodermic syringe to fill the capillary tube it is possible to do repeated dosages from a small quantity of stock solution. Evaporation of the stock solution and consequent concentration of insecticide does not occur in the syringe. The effect of any evaporation at the tip of the needle can be overcome by expelling a drop of liquid from the syringe immediately prior to recharging the capillary.

Orientation of Test Insects

The insects to be dosed are taken singly into the field of view of the microscope and opposed to the tip of the capillary tube. Insects are held by suction on the end of a piece of 3 mm diameter glass tubing about 25 mm long. The holding end is drawn out and its diameter flamed down to be small enough to stop the insect from being sucked down the tube. Suction is supplied from a small pump adjusted to work at 3–5 cm of Hg. Hand manipulation of the holder is facilitated by pressing it on to the piece of modelling clay on the microscopic stage. Final positioning of the insect at the tip of the capillary is adjusted under the microscope. The insect-holding tube is angled about 2·5 cm from its end (see Fig. 1) to make orientation of the insect on to the capillary tip easier.

Delivery of the Dose

The liquid in the capillary is expelled on to the insect by air pressure exerted by a rubber hand bulb connected to the open end of the capillary tube holder. Such an arrangement allows for sensitive pressure control and enables the transference of the liquid to be a positive and accurate procedure. Once the dose has been expelled, pressure is maintained until the insect is removed. This prevents partial refilling of the capillary from the liquid film on the insect's body.

Post-treatment of Insects

After treatment of each individual it is advisable that the amount of free liquid on the insect's body be reduced as quickly as possible. This prevents loss of liquid to a substratum as can occur when the insect is released from the suction holder into its cage. Accordingly the holder with the treated insect still attached is placed on a rack and a stream of air passed over the insect for a short time. The capillary is meanwhile refilled and cleaned ready for the next application. After drying, the treated insect is released by momentarily cutting off the suction and gently tapping the holder thus allowing the insect to fall into its cage. The cage is held in an atmosphere of CO_2 so that a batch of similarly treated individuals may be collected together.

APPLICATION OF APPARATUS

In this laboratory the apparatus has been used extensively on adult mites *Panonychus ulmi* (Koch) and *Tetranychus telarius* (L.) and on 1st instar larvae of *Tortix postvittana* Walk. and *Carpocapsa pomonella* (L.). Satisfactory reproducible log/probit regression lines have been obtained.

The whole procedure of treating an individual insect takes approximately one minute but with experience an operator can work at a faster rate.

ACKNOWLEDGMENTS

The author is indebted to the Radio Chemical Centre, Amersham, England, for suggesting the use of ^{32}P — tributyl phosphate in kerosene for the radiometric tests on the performance of the apparatus.

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THE INFLUENCE OF TEMPERATURE AND RELATIVE HUMIDITY ON THE DEVELOPMENT OF EGGS AND ON THE EFFECTIVENESS OF OVICIDES AGAINST *Tetranychus telarius* (L.) (ACARINA : TETRANYCHIDAE)

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Summary

An investigation has been made on the influence of temperature and relative humidity on eggs of *T. telarius* (L.). A new technique is described for carrying out such experiments involving the use of eggs laid on glass microscope slides rather than on leaf surfaces. A special aspirator is described for collecting the adult female mites used for oviposition. The egg laying cage used to obtain eggs on glass slides is also described. The relationship between temperature and egg incubation period is similar to that recorded by other workers and the mean incubation period is shown to range from 2·38 days at 32·5°C to 33·19 days at 11·5°C. Temperatures ranging from 16°C to 29·5°C are shown to have no influence on the toxicity of ovicides. Relative humidity except the extreme of 100% has little influence on the egg incubation time but is of major importance in determining the number of eggs which hatch. Both low and high humidities reduce the natural hatching percentage of eggs and 100% relative humidity delays hatching time so long that the embryo usually dies. The action of ovicides is greatly influenced by relative humidity. For five ovicides tested there is shown to be a marked decrease in the LC50 with an increase in relative humidity and over the range of relative humidities of 30% to 96% this decrease in LC50 is 14-fold for Chlorocide, 112 for Kelthane, 125 for Supona, 916 for Tedion and 2,000-fold for Chlorfenson.

Temperature is known to influence the speed of development of eggs and other stages of mites, and hence the distribution of ages of eggs in a population at any one time varies. The reaction of eggs of different ages to ovicides has been investigated and it is shown that for four sulphur-based ovicides tested (i.e., Chlorocide, Supona, Chlorfenson, and Tedion) the LC50 increases greatly as the age of eggs increases. This increase is so great for eggs just prior to hatch that eggs are considered to be virtually immune to these ovicides at that stage of their development. Similar tests with a non-sulphur ovicide, i.e., Kelthane, showed that there was no increase in LC50 with age of eggs.

The implications of these results in helping to explain the varying results obtained with ovicides in the field is discussed.

INTRODUCTION

Ovicides used for control of fruit-tree red spider mites in different localities in New Zealand have given varying results in the field. The causes of this variation are not well understood, but variations in environmental conditions may play some part. This paper records the effect of tempera-

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ture and relative humidity on the natural development of mite eggs and on the performance of ovicides in respect to egg mortality. Some of the work described here has been reported briefly by Harrison and Smith (1960).

METHODS

Of the three important red spider mites in New Zealand, *Tetranychus telarius* (L.) was chosen for the initial experiments because it can be reared on bean plants (*Phaseolus vulgaris* L.) in the glasshouse throughout the year.

Investigations on the effect of environmental conditions on mite eggs by other workers were carried out on eggs laid on leaves, e.g., Dosse (1952), Rodriquez (1953), Hussey *et al.* (1957). Because of the complicating effects produced by transpiration and radiant heating, it has not yet been possible, as far as is known, either to control or measure accurately the temperature and relative humidity immediately above the leaf surface. For this reason all experiments were conducted with eggs which were laid directly on to glass slides.

Egg Production

To obtain eggs on slides, adult female mites were first collected into batches of about 40 by means of an aspirator. Collection of these mites was readily accomplished if they were first shaken from their leaves on to sheets of white paper. Because of the inherent difficulty of handling mites, a special aspirator was developed (Fig. 1) which had a detachable container. Even so, successful collection depended on keeping the aspirator free from blockages due to webbing. This was produced during collection, especially if mites were left in the apparatus for any length of time. However, when in constant use as in a series of collections, blockages of this kind were infrequent.

After the required number of mites had been collected the aspirator was tapped on the bench so that all the mites dropped into the detachable tube. The tube was then removed and the mites tipped into an egg-laying cage. This consisted of two glass microscope slides separated by means of a paper spacer 0·015 in. thick with a 0·5 in. diameter hole and held together by rubber bands (Fig. 2).

Egg laying commenced within a few minutes of mites being introduced into the cage and within 4 hours at 25°C approximately 100 eggs were usually laid. Not all of these eggs were deposited on the glass slides. About 60% were laid within the webbing which was copiously produced within the cell. When the cage was opened the webbing came away with the paper spacer or remained on one slide. In the latter case it was removed either by blowing it away or lifting it off with a mounted needle. The number of eggs remaining on each glass slide averaged 20. The age of the eggs thus obtained was known within $\pm$ 2 hours. Variations in egg age could be reduced only at the expense of a lower yield of eggs per slide. Eggs obtained in this way were readily handled and counted.

Photo by S. A. Rumsey.

FIG. 1—Aspirator, developed for mite collection, with detachable container.

Ovicides

The materials in wettable powder formulations used in these experiments were:

Kelthane:	(1, 1 – Bis (*p*-chlorophenyl) – 2, 2, 2 – trichloroethanol)
Chlorocide:	(*p*-chlorobenzyl *p*-chlorophenyl sulphide)
Supona:	(Diphenyl sulphone)
Tedion:	(2, 4, 5, 4′ – Tetrachlorodiphenyl sulphone)
Chlorfenson:	(*p*-chlorophenyl *p*-chlorobenzene-sulphonate)

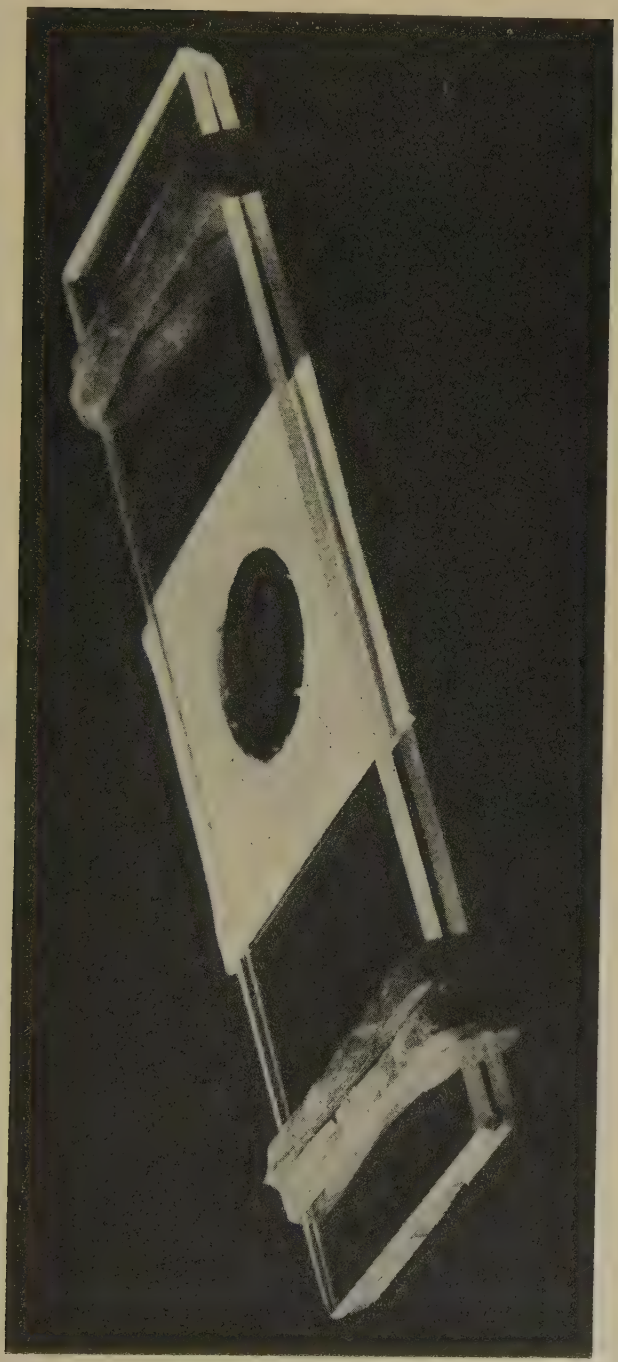

Photo by S. A. Rumsey.

FIG. 2—Mite egg-laying cage assembled.

The toxicity of these materials to mite eggs was determined by dipping the eggs on the glass slides in suspensions of known concentrations. The slides were held in the liquid for 30 seconds. Loss of eggs from the slides during the dipping process was negligible.

Environmental Control

Eggs were incubated under conditions of controlled temperature and relative humidity. The temperature fluctuations were not greater than $\pm$ 0·5°C. Constant relative humidities were obtained in small desiccators by the use of H_2SO_4 solutions.

RESULTS

Temperature

EFFECT ON EGG INCUBATION PERIOD

The egg incubation times at different temperatures and at constant relative humidity of 80% are given in Table 1. Means and standard deviations were calculated from log/probit regression lines.

TABLE 1—Relationship between Temperature and Incubation Period of Eggs of *T. telarius*

Temp. °C	Mean Incubation Period in Days	Standard Deviation
32·5	2·38	0·07
29·5	2·70	0·09
25·0	3·66	0·32
19·5	6·96	0·56
16·0	11·35	1·09
11·5	33·19	3·96

INFLUENCE ON TOXICITY OF OVICIDES

Freshly laid eggs were dipped in a range of concentrations of Kelthane, Tedion, and Chlorocide and then incubated in 80% relative humidity and at different temperatures. The range of concentrations chosen was known, from initial experiments, to result in mortality of eggs between 0% and 100% at 25°C. Results showed that there was no significant difference in toxicity of the three ovicides to eggs incubated in temperatures ranging from 16°C to 29·5°C.

Relative Humidity

EFFECT ON EGG INCUBATION PERIOD

The effect of relative humidity on the incubation period of eggs was determined in trials with eggs held in different constant relative humidities. In all tests a constant temperature of 25°C was maintained. Results are given in Table 2.

TABLE 2—Relationship between Relative Humidity and Incubation Period of Eggs of *T. telarius.*

% R.H.	Complete Egg Hatch in Days
30	5·2
50	4·8
70	4·8
90	4·2
94	5·2
96	5·8
98	7·0
100	Virtually no hatch

INFLUENCE OF RELATIVE HUMIDITY ON HATCH OF EGGS

Results are given in Table 3 of tests in which eggs were incubated at 25°C in relative humidities between 0% and 100%.

TABLE 3—Relationship between Relative Humidity and Hatch of Eggs of *T. telarius.*

Per cent R.H.	0	5	10	20	30	40–94	96	98	98·75	99	99·25	99·5	99·75	100
Per cent Hatch*	30	24	41	71	93	100	96	92	54	24	11	22	15	2

*A natural mortality of 7·5% has been allowed for.

INFLUENCE OF HIGH HUMIDITY ON EGG HATCH

Results of tests given in Table 3 showed that the higher relative humidities played a significant part in determining the natural hatch of eggs. This aspect has been further investigated by incubating eggs for portions of their incubation period in 100% relative humidity and then transferring them to 80% for the remaining period. All the tests were conducted at a temperature of 25°C. Results are given in Table 4.

TABLE 4—Hatch of Eggs of *T. telarius* Incubated Initially in 100% Relative Humidity and Transferred to 80% Relative Humidity

No. of Days in 100% R.H.	Per Cent Hatch of Eggs
1	96
2	98
3	86
4	96
5	96
6	93
7	82
8	64
9	20
10	16
11	0
12	0
13	9
14	0
No change to 80% R.H.	0

INFLUENCE OF RELATIVE HUMIDITY ON TOXICITY OF OVICIDES

Harrison and Smith (1960) showed that relative humidity had an important effect on the toxicity of Kelthane to eggs of *T. telarius*. Tests have now been completed with four additional ovicides. Freshly laid eggs were dipped in concentrations of ovicides (wettable powder formulations) and incubated in different constant relative humidities at 25°C. The concentration of ovicide which produced 50% mortality of eggs (LC50) was determined at each humidity by means of linear regression lines. Parallel lines were fitted for each ovicide, as the departures from parallelism were not significant. Results are given in Table 5.

TABLE 5—LC50 in Terms of Per Cent Active Material (wt/vol.) of Ovicides against Eggs of *T. telarius* Incubated at 25°C in Different Relative Humidities

Ovicide	Relative Humidity				
	30%	50%	70%	90%	96%
Kelthane	0·018	0·0093	0·0053	0·00035	0·00016
Chlorocide	0·020	0·026	0·013	0·0017	0·0014
Chlorfenson	0·15	0·19	0·0037	0·00023	0·000075
Supona	0·11	0·090	0·047	0·0058	0·00088
Tedion	0·011	0·0053	0·00036	0·000027	0·000012

EFFECT OF OVICIDES ON EGGS OF DIFFERENT AGES

The toxicity of the five ovicides has been determined for eggs of different ages to find if age of eggs influences the action of ovicides. This was done by holding eggs at 25°C and dipping some of them in a range of concentrations on each successive day until the day before hatching was due to commence. The treated eggs were then held at 25°C in 80% relative humidity until hatch was complete. The calculated LC50's are given in Table 6.

TABLE 6—LC50 of Ovicides in Terms of Per Cent Active Ingredient (wt/vol.) against Eggs of *T. telarius* of Different Ages.

Ovicide	Age of Eggs at Dipping			
	0 hr	24 hr	48 hr	72 hr
Kelthane	0·0006	0·0006	0·0006	0·0006
Chlorocide	0·0016	0·0029	0·0077	0·065
Chlorfenson	0·00041	0·00057	0·0024	0·19
Supona	0·0012	0·0015	0·0024	0·0071
Tedion	0·000088	0·00029	0·00077	0·015

Tests carried out with ovicides on eggs immediately prior to hatch, gave such varying results in respect to regression line slopes that they were considered too unreliable for publication.

DISCUSSION

The effect of temperature in determining the duration of the incubation period of *T. telarius* and other closely related species has been shown by other workers, e.g., Cagle (1949), Dosse (1952), Bravenboer (1959) using eggs laid on leaves. However, because the eggs were on leaves, the exact temperature to which they were subjected was uncertain. The results given in Table 1, although similar in trend to those recorded by these other workers, show a more precise relationship between temperature and incubation period, the mean and standard deviation being given.

It was found that temperature does not influence the action of ovicides at least within the range of 16°C to 29·5°C. However, as temperature so greatly influences the duration of the egg incubation period and all other facets of the life history of mites (Linke (1953) and Bravenboer (1959)), the distribution of egg ages in a population at any particular moment, in the field, will be governed by the temperatures of the previous days or weeks. Thus an egg population may contain at one time a predominance of older eggs and at another a predominance of younger eggs. The relationship between age of eggs and toxicity of ovicides is therefore of importance. Meltzer and Dietvorst (1957) recorded that the susceptibility of eggs to Tedion was the same for eggs 0 to 3 days old and that a difference was only shown with 4-day-old eggs which were almost due to hatch. The results presented here show (Table 6) that with all the sulphur-based ovicides there is a major variation in susceptibility to ovicides according to age of eggs. With these ovicides there was an increase in LC50 with an increase in the age of eggs. For eggs 0 to 2 days old incubated at 25°C this increase in LC50 was relatively regular but for 3-day-old eggs the increase was considerably greater. Further, it was found from tests on eggs immediately prior to hatch that the amount of ovicide required to obtain a reasonable kill was so great as to make eggs at this stage of their development virtually immune to these chemicals.

The non-sulphur ovicide, Kelthane, behaved differently from the sulphur ovicides. Table 6 shows that egg age had no bearing on the toxicity of this material.

Relative humidity is of great importance to egg hatch and influences the action of ovicides to a marked degree. Table 2 shows that the actual incubation period of eggs is not influenced greatly by different relative humidities and Linke (1953) using *T. althaeae* showed that there was no difference in incubation period when eggs were incubated in 55%, 70%, or 100% relative humidity. However, the effect of relative humidity on the percentage hatch of eggs is important. This is shown in Table 3. In this experiment, eggs held in relative humidities ranging from 30% to 98% hatched normally with a low natural mortality under 10%. Below 30% relative humidity some desiccation of eggs takes place and in the driest conditions of 0% there was a 70% mortality of eggs. In the higher humidities of above 98% there was a high mortality possibly due to drowning of the embryo, while at saturation practically all eggs fail to hatch. This aspect of the effect of relative humidity on hatch of eggs was also investigated by Boudreaux (1958). His results were that hatching was

apparently not affected by extremes of humidity, i.e., 95%–100% and 0%–35%. Because his experiments were with eggs on leaf surfaces his recorded range of relative humidity 0%–35% is suspect and the actual humidity surrounding the eggs could not have been known and may easily have been higher than 35%. The effect on egg hatch produced by a change of relative humidity during the incubation period of eggs is illustrated by results given in Table 4. These results show that death of the embryo, due to exposure of eggs continuously to saturated atmospheres, occurred at about 11 days at 25°C but some mortality was occurring as early as 7 days. Under the temperature conditions of these tests egg hatch could have been expected between 4 and 5 days and the table shows that when embryonic development was virtually completed, a change to 80% relative humidity allowed the embryo to emerge.

The most significant effect of relative humidity is the influence it has on the toxicity of ovicides. With the five ovicides tested (Table 5) there was a marked increase in toxicity with an increase in the relative humidity. The table shows that increases were a 14-fold increase for Chloricide, 112 for Kelthane, 125 for Supona, 916 for Tedion, and 2,000 for Chlorfenson, in the 30% to 96% range of humidities.

CONCLUSION

For purposes of comparison a theoretical model of a mite egg population can be envisaged where, due to steady temperatures, the egg population could consist of equal numbers of eggs of all ages, and where, due to leaf transpiration only, the eggs could be subjected to a constant relative humidity. Our results show that an application of ovicide on this population would give only a fair control.

If this situation were altered so that there was a population of predominantly young eggs, as would occur when a period of relatively high temperatures had been preceded by several days of relatively low temperatures, control would be improved. A similar improvement in control could be expected if a period of high relative humidity on the leaf surfaces persisted after spraying, as might obtain during wet weather. If these two conditions coincided, very good control would be expected.

In conditions of low temperature following a period of high temperatures or conditions of low relative humidity brought about by dry windy weather, the opposite would occur, giving much less control than that envisaged for the model. Both these conditions occurring together would result in poor control or even no control at all.

In addition to their purely ovicidal properties, ovicides have, in some cases, a larvacidal effect and a sterilising effect on females. Although the work in this paper deals exclusively with the ovicidal effect it shows that even this property, when influenced by fluctuations of temperature and relative humidity could account for much of the variations met with in the field.

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THE EFFECTS OF BIOTIN AND BIOTIN ANALOGUES ON SOME METABOLIC PROCESSES OF MICRO-ORGANISMS

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Summary

Biotin-deficient cells of *Propionibacterium pentosaceum* have a reduced ability to decarboxylate succinate. The activity is restored by biotin, dethiobiotin or oxybiotin, but further reduced by biotin homologues. The cells also have a reduced ability to ferment glucose, but can be reactivated by biotin or dethiobiotin.

Saccharomyces cerevisiae grown under biotin-deficient conditions ferments glucose or fructose at a low rate. Additions of biotin or dethiobiotin are stimulatory: norbiotin and homobiotin are inhibitory. The fermentation of phosphorylated hexoses by the biotin-deficient organism is not much reduced and biotin additions are without effect. Glucose fermentation is greatly stimulated by the addition of crystalline hexokinase. A combination of hexokinase and biotin, or dethiobiotin, is even more effective. Combinations of hexokinase and biotin homologues, however, are only poor stimulants. The hexokinase activity of cell-free preparations of the deficient yeast is not significantly affected by biotin or biotin analogues.

Bacillus macerans accumulates alpha-keto acids in the medium. Much more alpha-keto acids accumulate from biotin-deficient cells than from normal. Glucose oxidation and pyruvate oxidation by the deficient cells are greatly reduced but can be partially restored by the addition of biotin. Pyruvate oxidation can be restored by additions of ATP or DPN.

These results demonstrate a variety of roles for biotin in microbial metabolism. A study of the coenzyme content of biotin deficient yeast indicates that many of the abnormalities of the deficient organisms can be explained in terms of poor synthesis of coenzymes.

Abbreviations used in the text:

ATP	adenosine triphosphate
DPN	diphosphopyridine nucleotide
TPN	triphosphopyridine nucleotide

INTRODUCTION

Biotin has been shown to affect numerous metabolic processes of micro-organisms. Probably the best investigated biotin-dependent reactions are carboxylations and decarboxylations. For example Lardy *et al.* (1947) have shown that under certain conditions the growth of *Lactobacillus arabinosus* is stimulated by bicarbonate. The stimulation, however, occurs only in the presence of biotin. More specifically it was demonstrated by Lichstein and Umbreit (1947) that the oxalacetic carboxylase activity of *Escherichia coli* preparations is greatly decreased in biotin-deficient conditions. Wessman and Werkman (1950) have reported similar findings for lysed preparations of *Micrococcus lysodeikticus*. According to Cantino (1953) and Cantino and

Hyatt (1953) biotin-deficiency reduces the biosynthesis of alpha-ketoglutarate by the aquatic phycomycete *Blastocladiella emersonii*. Similar findings for mammalian liver were published by Shive and Rogers (1947), while Katsuki (1959) has reported that alpha-ketoglutarate, together with other alpha-keto acids, accumulate in the medium of biotin-deficient *Piricularia oryzae*. Succinate decarboxylation by *Propionibacterium pentosaceum* requires biotin (Delwiche, 1950). Biotin also is involved in the formation of long chain fatty acids (Wakil *et al.*, 1958), malate biosynthesis (Ochoa, *et al.*, 1942), the carboxylation of beta-hydroxy-isovaleryl-coenzyme A (Woessner, *et al.*, 1958) and purine synthesis (Moat et al., 1956). All these processes probably involve the fixation or removal of carbon dioxide.

At the present time there is no universally accepted hypothesis of the mode of action of biotin in these reactions. From studies of the carboxylation of beta-methyl crotonyl coenzyme A, Lynen (1959) and Lynen *et al.* (1959) have proposed the scheme outlined in Fig. 1.

$$ATP + B{-}E \rightleftharpoons ADP{-}B{-}E + P_i$$

$$ADP{-}B{-}E + CO_2 \rightleftharpoons ADP + CO_2{-}B{-}E$$

$$CO_2{-}B{-}E + B \rightleftharpoons CO_2{-}B + B{-}E$$

ATP = adenosine triphosphate

B—E = biotin—enzyme complex

ADP—B—E = adenosine diphosphate—biotin—enzyme complex

Pi = inorganic orthophosphate

ADP = adenosine diphosphate

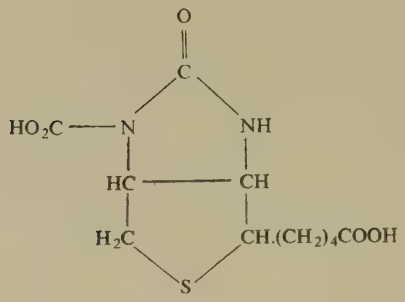
CO_2—B—E = carbon dioxide—biotin—enzyme complex

CO_2—B = carbon dioxide-biotin compound

FIG. 1

The suggested structure of the biotin-carbon-dioxide compound is given in Fig. 2.

This compound is said to be very labile in dilute acid, but to be stable in alkaline or neutral media. It is apparent, however, that the hypothetical scheme depends on the presence of bound-biotin in the enzyme catalysing the carboxylation of beta-methylcrotonyl-coenzyme A. While the presence of physiologically significant amounts of bound-biotin in this enzyme, and also in propionyl carboxylase (Kaziro *et al.*, 1960) has been demonstrated there is evidence to show that many other biotin-dependent enzymes do not



biotin—carbon dioxide compound

FIG. 2

contain more than traces of microbiologically detectable biotin (Briggs, 1960a; Ravel, *et al.* 1959; Ochoa, *et al.*, 1942; Hamilton and Westheimer, 1959). Hence it must be concluded that either biotin does not function in the same manner in all biotin-dependent systems, or that microbiological assays cannot detect the active form of enzyme-bound biotin.

It is known that various structural analogues of biotin are active inhibitors of the growth of certain micro-organisms (Briggs, 1960b) while inhibition of specific metabolic processes has been demonstrated (Ajl *et al.*, 1950; Sundaram *et al.*, 1954; Tirunarayanan *et al.*, 1954). Hence if biotin functions as a coenzyme by the mechanism postulated in Fig. 1, it is probable that biotin analogues will compete with biotin for the displacement of the biotin-CO_2 compound from the CO_2-biotin-enzyme complex. Hence it should be possible to inhibit biotin-dependent processes by the use of biotin analogues both *in vivo* and in purified systems.

The present studies were conducted to determine the effects of biotin and biotin analogues on succinate decarboxylation, alpha-ketoacid biosynthesis, hexose fermentation, and pyruvate oxidation by a variety of micro-organisms.

METHODS AND MATERIALS

Micro-organisms

The micro-organisms used in this study were *Propionibacterium pentosaceum* (E 214), *Bacillus macerans* Schardinger 3482, and *Saccharomyces cerevisiae* Fleishmann 139.

P. pentosaceum was grown in the synthetic medium given in Table 1. This medium is based on that of Delwiche (1950). The biotin content in some experiments was 4 mμg per ml and in other 1 mμg per ml. The medium was adjusted to pH 6·8. Inoculation was with cells (washed four times in sterile distilled water) from a 48 hour culture in 25 ml of a medium containing 1% glucose, 1% yeast extract, 1% peptone, and 0·5% dipotassium phosphate. Active cell suspensions were obtained by growth in the synthetic

medium for 2 to 3 days at 30°C under stationary conditions. Cells were recovered from the medium by centrifugation and were re-suspended in distilled water for use in the Warburg apparatus.

TABLE 1—Synthetic Medium for *P. pentosaceum*

Compound	mg/litre
D-glucose (AR)	10,000
sodium acetate $3H_2O$	8,000
casein hydrolyzate (vitamin-free)	5,000
dipotassium phosphate $3H_2O$	4,000
sodium chloride	4,000
magnesium sulphate $7H_2O$	250
sodium thioglycolate	200
L-cystine	50
L-tryptophan	50
Ferrous sulphate $7H_2O$	15
manganese sulphate $4H_2O$	4
adenine	10
guanine	10
uracil	10
xanthine	10
calcium pantothenate	1
p-aminobenzoic acid	1
thiamine hydrochloride	1

B. macerans was grown in the synthetic medium given in Table 2; a modification of the medium of Knight and Proom (1950). When grown under biotin-deficient conditions the biotin content of the medium was 8 mμg per litre. In other experiments the concentration was 2,000 mμg biotin per litre. The medium was adjusted to pH 7·3.

TABLE 2—Synthetic Medium for *B. macerans*

Compound	mg/litre
D-glucose (AR)	30,000
diammonium phosphate	5,000
dipotassium phosphate $3H_2O$	2,000
sodium glutamate	2,000
potassium dihydrogen phosphate	1,500
sodium chloride	1,000
magnesium sulphate $7H_2O$	500
calcium chloride $2H_2O$	300
manganese sulphate $4H_2O$	40
ferrous sulphate $7H_2O$	25
ammonium molybdate	20
thiamine hydrochloride	10

Inoculation was with cells (washed three times in sterile 1/15 M phosphate buffer pH 7·3) obtained from a subculture in the biotin-deficient medium for 2 days at 37°. Active cell suspensions were obtained by growth

of the bacteria in the synthetic medium for 2 days at 37°C under stationary conditions. Cells were recovered from the medium by centrifugation and were suspended in phosphate buffer pH 7·3, after two washings, for use in the Warburg apparatus.

S. cerevisiae was carried as slant cultures on Sabouraud's agar at room temperature. Cultures were grown in the medium given in Table 3 which is based on that of Snell *et al.* (1940). Biotin-deficient cells were obtained by growth in a medium containing 25 mμg of d-biotin per litre. Normal cells were harvested from a medium containing 1 μg of biotin per litre. In both cases the culture was grown from 18–20 hours at 32°C and cells collected by centrifugation. After washing three times with distilled water the cells were air-dried for the hexose fermentation experiments, or used immediately for the cofactor assays.

TABLE 3—Synthetic Medium for *S. cerevisiae*

Compound	mg/litre
sucrose (AR)	34,000
ammonium sulphate	5,000
potassium dihydrogen phosphate	3,500
casein hydrolyzate (vitamin-free)	1,000
L-aspartic acid	170
magnesium sulphate $7H_2O$	450
calcium chloride $2H_2O$	450
	p.p.m.
inositol	90
boric acid	18
zinc sulphate	18
thiamine hydrochloride	10
pyridoxine hydrochloride	10
ferric chloride	9
calcium pantothenate	5
manganese chloride	2
potassium iodide	2
cupric sulphate $5H_2O$	2

Determination of Succinate Decarboxylation

Succinate decarboxylase activity of biotin-deficient *P. pentosaceum* was determined by standard Warburg techniques by manometric measurement of carbon dioxide evolution. The Warburg vessels contained 1·0 ml of the cell suspension (20 mg) in the main compartment. The side arm contained 0·2 ml of M/5 succinate. In some experiments 0·2 ml of a solution of biotin, or biotin analogue at various concentrations, in M/10 phosphate buffer, pH 5·2, was added to the main compartment.

Incubation was at 30°C for 60 minutes after tipping the substrate. Manometric readings were taken every 10 minutes.

Determination of Alpha-keto Acids

Washed cells of biotin-deficient and normal cultures of *B. macerans* were separately suspended in 10 ml M/10 phosphate buffer, pH 7·3, containing 30 g D-glucose per litre. The suspensions were incubated at 37°C for 120 min. with continuous aeration. Cells were removed from the medium by centrifugation and the accumulated alpha-keto acids determined by the 2, 4-dinitrophenyl-hydrazone method of Katsuki (1959). Only total alpha-keto acids and alpha-ketoglutarate were specifically determined. The difference between these results was assumed to be pyruvate.

Determination of Hexose Fermentation and Oxidation

Hexose fermentation by biotin-deficient cultures of *P. pentosaceum* and *S. cerevisiae* and hexose oxidation by *B. macerans* were determined by standard Warburg techniques.

Fermentation of glucose by *P. pentosaceum* was followed by carbon dioxide production. Warburg vessels contained 1·0 ml of the biotin-deficient cells, suspended in distilled water, in the main compartment. The side-arm contain 0·2 ml of a solution of D-glucose to give a final concentration of 0·003 M. The total volume was 3·0 ml and was achieved by the addition of 0·04 M phosphate buffer, pH 5·2. In some experiments 0·2 ml of a solution of biotin, or biotin analogue, at various concentrations, was added. The gas phase was nitrogen. Incubation was for one hour at 30°C.

Control experiments were conducted without substrate.

Hexose fermentation by *S. cerevisiae* was determined by the use of air-dried cells (Meyerhof, 1949) in a Warburg apparatus. Carbon dioxide production was determined at 37°C with 95% nitrogen – 5% carbon dioxide as the gas phase. Each vessel contained 20 mg dry-weight of biotin-deficient cells; 1·0 ml M/10 phosphate buffer, pH 6·5, and 1·0 mg magnesium sulphate in the main compartment. The side-arm contained 20 micro-moles of either D-glucose or D-fructose; 20 mg acetaldehyde; 1·0 mg ATP; 1·0 mg DPN, and 0·01 mg of cocarboxylase. Biotin or analogue, when present, was preincubated with the cells for 30 minutes. Endogenous rates were determined with vessels containing cells, all cofactors including biotin, but no substrates.

To determine the effects of hexokinase on the fermentation, in some experiments 1·0 mg of a crystalline sample of the enzyme was preincubated with the cells for 30 minutes before the addition of the substrate and cofactors from the side arm.

Glucose oxidation by normal and biotin-deficient cultures of *B. macerans* was compared also by Warburg techniques. The main compartments of the vessels contained equal weights (12 mg) of cells in M/15 phosphate buffer and 1 micromole of manganese sulphate. The side arm contained 50 micro-moles of D-glucose. The centre-well contained 0·2 ml of 40% potassium hydroxide solution on paper. The final total volume was 3·0 ml. The gas phase was air.

Determination of Pyruvate Oxidation

Pyruvate oxidation by normal and biotin-deficient cells of *B. macerans* was determined under similar conditions to those described above for the measurement of glucose oxidation. The glucose, however, was replaced by 50 micromoles of sodium pyruvate.

Determination of Cofactors in Yeast

Normal and biotin-deficient cultures were grown for several days and then harvested by centrifugation. They were assayed for cofactors as follows: Coenzyme A by the arsenolysis of acetylphosphate (Novelli, 1955), ATP by a chromatographic assay (Schmitz, 1954) and DPN by the alcohol dehydrogenase assay (Kornberg, 1950).

Source of Cofactors

d-Biotin, dl-oxybiotin and d-dethiobiotin were purchased from Nutritional Biochemicals Corporation; d-homobiotin and d-norbiotin were obtained from Hoffman-La Roche Inc. Sigma Chemical Company supplied all other cofactors and enzymes.

RESULTS

Effect of Biotin and Biotin Analogues on Succinate Decarboxylation

Biotin-deficient cells of *P. pentosaceum* have a greatly reduced ability to decarboxylate succinic acid as compared to normal cells. A comparison of the succinate decarboxylase activity of cells (grown in a medium containing 4 mμg of biotin per ml) to which biotin had been added with untreated cells is given in Table 4. It is clear that the decarboxylase activity is considerably stimulated by the biotin. The stimulation, moreover, was apparent within the first ten minutes even though the deficient cells had been in contact with the biotin for only about 15 minutes. To determine the immediate effects of biotin on the reaction, the experiment was repeated with the biotin solution in a second arm of the Warburg flask. The reaction was initiated by tipping the substrate and the biotin was added after 30 minutes. The results (line 5 of Table 4) again show that biotin is able to stimulate the reaction within a few minutes and no long preincubation period is necessary.

TABLE 4—Effect of Biotin on Succinate Decarboxylation by Biotin-deficient *P. pentosaceum*

Conditions	μl Carbon Dioxide Evolved			
	10 min	20 min	40 min	60 min
1. endogenous deficient cells	7	9	12	15
2. deficient cells + succinate	11	22	44	69
3. deficient cells + succinate + biotin	20	44	98	141
4. endogenous deficient cells + biotin	7	10	13	17
5. deficient cells + succinate + biotin*	11	22	58	120

*tipped after 30 min.

The effects of various biotin analogues on succinate decarboxylation by the deficient cells are recorded in Table 5. Biotin and oxybiotin appear to have similar activities for the system, but dethiobiotin has greater activity than either at concentrations less than 10^{-2} μg. Both these analogues involve alterations to the tetrahydrothiophene ring of the biotin molecule. The analogues nor-biotin and homobiotin proved to be inhibitors (see Table 6) and differ from biotin by the length of the side-chain. In these experiments the biotin-deficient cells were taken from a medium containing 1 μg of biotin per ml.

TABLE 5—Stimulatory Effect of Biotin Analogues on Succinate Decarboxylation by Biotin-deficient *P. pentosaceum*

Conditions	Analogue	Concn. μg	Carbon Dioxide Evolved μl/60 min
1. endogenous deficient cells	—	—	15
2. deficient cells + succinate	—	—	69
3. deficient cells + succinate	biotin	4	140
4. deficient cells	biotin	4	18
5. deficient cells + succinate	biotin	1	136
6. deficient cells + succinate	biotin	0·1	118
7. deficient cells + succinate	biotin	0·01	25
8. deficient cells + succinate	dethiobiotin	1	139
9. deficient cells + succinate	dethiobiotin	0·1	119
10. deficient cells + succinate	dethiobiotin	0·01	90
11. deficient cells + succinate	dethiobiotin	0·001	21
12. deficient cells + succinate	oxybiotin	1	120
13. deficient cells + succinate	oxybiotin	0·1	106
14. deficient cells + succinate	oxybiotin	0·01	15

TABLE 6—Inhibitory Effect of Biotin Analogues on Succinate Decarboxylation by Biotin-deficient *P. pentosaceum*

Conditions	Analogue	Concn. μg	Carbon Dioxide Evolved μl/60 min
1. endogenous deficient cells	—	—	7
2. deficient cells + succinate	—	—	34
3. deficient cells + succinate	biotin	1	135
4. deficient cells + succinate	norbiotin	10	18
5. deficient cells + succinate	homobiotin	10	15

Effects of Biotin on Accumulation of Alpha-keto Acids by B. macerans

Alpha-keto acids were detected in the buffered glucose solution in which a culture of *B. macerans* had been incubated with aeration. Considerably more alpha-keto acids were produced by the deficient than the normal cells. The results given in Table 7 are the mean of several determinations. Variations in concentration of up to 50% were noted in individual experiments.

TABLE 7—Alpha-keto Acid Content of *B. macerans* Medium

Conditions	Alpha-keto Acid	Concentration mμg/μg Cells/hour
normal cells	pyruvate	16
deficient cells	pyruvate	73
normal cells	alpha-ketoglutarate	2
deficient cells	alpha-ketoglutarate	13

Effects of Biotin and Biotin Analogues on Hexose Fermentation

Biotin-deficient cultures of *P. pentosaceum* ferment D-glucose under anaerobic conditions. The rate of fermentation, however, can be increased by biotin or dethiobiotin and decreased by homobiotin (see Table 8).

TABLE 8—Effects of Biotin and Biotin Analogues on Glucose Fermentation by Biotin-deficient *P. pentosaceum*

Conditions	Analogue	Concn. μg	Carbon Dioxide Evolved μl/60 min
Endogenous cells	—	—	5
Cells + D-glucose	—	—	21
Cells + D-glucose	biotin	10	64
Cells + D-glucose	dethiobiotin	10	64
Cells + D-glucose	homobiotin	10	12

Similar effects were observed with hexose fermentation by biotin-deficient yeast. Here a more detailed study was made by observing the effects of biotin and biotin analogues on the fermentation of both D-glucose and D-fructose. Results are given in Table 9.

TABLE 9—Effects of Biotin and Biotin Analogues of Hexose Fermentation by Biotin-deficient *S. cerevisiae*

Hexose	Analogue	Concn. μg	Carbon Dioxide Evolved μl/60 min
Endogenous cells	—	—	15
D-glucose	—	—	45
D-glucose	biotin	3	102
D-glucose	dethiobiotin	3	105
D-glucose	homobiotin	10	29
D-glucose	norbiotin	10	39
D-fructose	—	—	39
D-fructose	biotin	3	91
D-fructose	dethiobiotin	3	92
D-fructose	homobiotin	10	27
D-fructose	norbiotin	10	36

From studies of the fermentation of hexose analogues by yeast Williams *et al.* (1957) have suggested that biotin is involved in the hexokinase reaction. Supporting evidence for this hypothesis has come from Strauss and Moat (1958). To investigate the possibility of involvement of biotin in hexokinase reactions a comparison was made of the fermentation of Embden-Meyerhof pathway intermediates by normal and biotin-deficient yeast cells. The results (Table 10) clearly demonstrate that the fermentation of only the unphosphorylated hexoses is affected by the deficiency.

TABLE 10—Fermentation of Embden-Meyerhof Pathway Intermediates by Normal and Biotin-deficient Yeast

Compound	Additions (10 μg)	μl Carbon Dioxide/60 min	
		Normal	Biotin-deficient
Glucose	none	125	57
Glucose	biotin	125	105
Glucose-6-phosphate	none	121	115
Glucose-6-phosphate	biotin	122	114
Fructose-6-phosphate	none	64	52
Fructose-6-phosphate	biotin	65	53

In other experiments the effects of 1 mg of crystalline hexokinase, alone and in combination with biotin and biotin analogues, on glucose fermentation by the dried, biotin-deficient yeast were determined. The results (Table 11) demonstrate that the fermentation is stimulated by the enzyme and that this stimulation is enhanced by the addition of biotin or dethiobiotin, though reduced by biotin homologues.

TABLE 11—Effects of Hexokinase and Biotin Analogues on Glucose Fermentation by Biotin-deficient Yeast

Conditions	Additions	μg Conc.	Carbon Dioxide Evolved μl/60 min
Endogenous cells	—	—	15
Endogenous cells + hexokinase	—	—	16
Cells + D-glucose	—	—	48
Cells + D-glucose + hexokinase	—	—	145
Cells + D-glucose + hexokinase	biotin	3	195
Cells + D-glucose + hexokinase	dethiobiotin	3	194
Cells + D-glucose + hexokinase	norbiotin	10	111
Cells + D-glucose + hexokinase	homobiotin	10	93

In a previous paper (Briggs, 1960a) it has been reported that biotin and biotin analogues are without affect on the activity of crystalline hexokinase assayed *in vitro*. Similarly Williams *et al.* (1957) were unable to stimulate the hexokinase activity of cell-free extracts of biotin-deficient yeast. However, Strauss and Moat (1958) have reported a stimulation of hexokinase by biotin in cell-free extracts of deficient yeast prepared by a Nossal dis-

integrator but not in peparations from a sonic oscillator. These latter two experiments were repeated in the present study. Hexokinase was assayed by measuring TPN reduction at 340 mμ in a Beckman spectrophotometer with a coupled hexokinase-glucose-6-phosphate dehydrogenase system (details as given by Strauss and Moat, 1958). Contrary to the previous study the present results (Table 12) do not demonstrate any significant stimulation by biotin: nor do biotin analogues exhibit any inhibitory effect on preparations from either a Nossal disintegrator or a sonic oscillator.

TABLE 12—Effect of Biotin and Biotin Analogues on the Coupled Hexokinase-glucose-6-phosphate Dehydrogenase System of Cell-free Extracts of Biotin-deficient Yeast

Preparation*	Additions**	TPN Reduction at 340 mμ OD × 10^3 2 min	4 min
Nossal extract + glucose	—	145	315
Nossal extract + glucose	biotin	150	324
Nossal extract + glucose	dethiobiotin	148	318
Nossal extract + glucose	homobiotin	144	310
Sonic extract + glucose	—	147	319
Sonic extract + glucose	biotin	149	324
Sonic extract + glucose	dethiobiotin	150	325
Sonic extract + glucose	homobiotin	144	312

*1·5 mg protein per cuvette

**3 μg biotin or dethiobiotin, 10 μg homobiotin; no preincubation

Effects of Biotin on Glucose Oxidation by B. macerans

A comparison of the rates of glucose oxidation by normal and biotin-deficient cells of *B. macerans* clearly demonstrates that the oxidation is considerably impaired by the lack of biotin. Moreover, while additions of biotin to the normal cells are without effect, biotin stimulates the oxidation by the deficient cells about 100%. Results are given in Table 13.

TABLE 13—Effects of Biotin on Glucose Oxidation by *B. macerans*

Conditions	Oxygen Uptake, μl/60 min
endogenous biotin-deficient cells	24
biotin-deficient cells + glucose	95
biotin-deficient cells + glucose + 3 μg biotin	194
endogenous normal cells	26
normal cells + glucose	345
normal cells + glucose + 3 μg biotin	345

Effect of Various Cofactors on Pyruvate Oxidation by B. macerans

It is apparent that glucose oxidation could be impaired by biotin-deficiency at various sites other than the initial phosphorylation. A further site is clearly illustrated by the results given in Table 14 from which it is apparent that biotin-deficient cells have a greatly reduced ability to oxidise pyruvate.

TABLE 14—Effects of Biotin on Pyruvate Oxidation by *B. macerans*

Conditions	Oxygen Uptake, μl/60 min
Endogenous biotin-deficient cells	24
biotin-deficient cells + pyruvate	29
biotin-deficient cells + pyruvate + 3 μg biotin	105
endogenous normal cells	26
normal cells + pyruvate	130
normal cells + pyruvate + 3 μg biotin	131

The study was extended by testing the effects of various cofactors on the oxidation. The results (Table 15) show that the addition of ATP is sufficient in itself to restore the rate of oxidation by the deficient cells to that of normal cells. DPN has a similar effect; indicating that the biotin-deficient cells are probably deficient also in ATP and DPN.

TABLE 15—Effects of Various Cofactors on Pyruvate Oxidation by *B. macerans*

Additions*	Oxygen Uptake, μl/60 min	
	Biotin-deficient	Normal
None	24	128
biotin	102	129
ATP	125	130
ATP + biotin	127	134
DPN	129	128
DPN + biotin	130	132
DPN + ATP	130	131
DPN + ATP + biotin	132	134

*Concentration of cofactors: biotin 1 μg ATP 5 μM DPN 5 μM.

Coenzyme Content of Yeast

The results given in Table 16 demonstrate that the coenzyme A content of normal and biotin-deficient yeast is very similar. However, the deficient organisms contain much less DPN and ATP than the normal.

TABLE 16—Coenzyme Content of *S. cerevisiae*

Coenzyme	Concentration: Fresh Tissue	
	Normal	Biotin-deficient
Coenzyme A[a]	54	55
ATP[b]	20	3
DPN[c]	1·22	0·24

(a) units/g (b) micromoles/100 g (c) mg/g

CONCLUSIONS

The results presented above of the investigation of the metabolism of biotin-deficient micro-organisms indicate that succinate decarboxylation, hexose phosphorylation, and pyruvate oxidation are reduced in the absence of biotin. The role of biotin in these processes is difficult to determine from the information available. The impairment of pyruvate oxidation presumably indicates an inhibition of the Krebs' tricarboxylic acid cycle. This is to be expected if the alpha-ketoglutarate dehydrogenase series of reactions is absent. The observed accumulation of alpha-ketoglutarate may be explained in this manner.

It would seem reasonable to assume that the lack of oxidative metabolism through the Krebs' cycle is the most important derangement of metabolism in biotin-deficiency for little ATP can be produced. In the absence of ATP many other reactions will be slowed or absent, i.e., biosynthesis of pyridine nucleotides. Hence biotin-deficiency must be accompanied by a deficiency of DPN, TPN, and ATP.

The role of biotin in the hexokinase reaction is puzzling. Biotin and biotin analogues have no effect on the reaction *in vitro* and no apparently significant effect on the reaction in cell-free preparations. Yet with the air-dried cells a notable stimulation by biotin and inhibition by biotin homologues is apparent.

In the absence of any agreement about the mode of action of biotin in enzyme reactions, the results can be considered from the viewpoint of the following three alternative hypotheses:

1. Biotin is a coenzyme.
2. Biotin is a part of a larger coenzyme molecule.
3. Biotin is essential for the synthesis of some enzyme molecules.

The first experiments in which biotin and analogues stimulated and inhibited glucose and fructose fermentation by biotin-deficient yeast demonstrate merely that biotin is concerned somewhere in glycolysis, but in combination with the other experiments which specifically indicate hexokinase to be biotin-dependent, they are of particular significance. The equal stimulation of fermentation by biotin and dethiobiotin is not surprising for it

has been shown that yeast can convert dethiobiotin to biotin. During the 30 minute incubation period this reaction was probably carried to completion. The nature of the stimulation is not clear from this experiment as it can be equally well explained on any of the three hypotheses of the mode of action of biotin. Either the synthesis of new hexokinase enzyme was promoted, or inactive enzyme already present was activated. The inhibition produced by the biotin homologues, however, is of greater importance. On the enzyme synthesis hypothesis it would be expected that biotin antagonists would inhibit the synthesis of new enzyme molecules, but would presumably be without effect on the activity of enzyme already present. Hence the observed inhibition of the basal rate of fermentation by the biotin-deficient cells can be regarded as evidence against this hypothesis. The inhibition, however, is compatible with both coenzyme hypotheses.

The second experiment, in which additions of hexokinase were observed to stimulate glucose fermentation by the deficient cells, is of greater significance. As normal cells are not stimulated in this way, it is reasonable to assume that the deficient cells contain either less hexokinase than the normal, or that their hexokinase is inactive. This initial observation is consequently compatible with all three hypotheses. Similarly the stimulation produced by a combination of hexokinase and biotin or dethiobiotin could be due either to increased hexokinase synthesis by the cells or the activation of hexokinase already present. The inhibition, however, again appears to be explicable on only a coenzyme hypothesis, for if biotin is concerned in enzyme synthesis then the analogues should not inhibit active hexokinase. This hypothetical hexokinase coenzyme is unlikely to be the biotin-CO_2 complex identified in carboxylation systems.

Yet the absence of microbiologically detectable biotin from highly active pure samples of hexokinase remains to be explained.

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AN EVALUATION OF THE METABOLIC STATUS OF BIOTIN

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Summary

A detailed review of the literature on biotin is presented. A list of biotin-requiring organisms is included and the effects of biotin on all aspects of intermediary metabolism are discussed. From a consideration of this evidence it is concluded that none of the hypothetical schemes to account for the mode of action of biotin in enzyme systems is completely satisfactory. A coenzyme function in some carboxylation systems has been established, but other biotin-dependent enzymes, including some carboxylases, do not appear to require a biotin-coenzyme. Biotin may play some role in the biosynthesis of these enzymes.

Abbreviations used in the text:

ATP	=	Adenosine – 5′ – triphosphate
ADP	=	Adenosine – 5′ – diphosphate
AMP	=	Adenosine – 5′ – monophosphate
DPN	=	Diphosphopyridine nucleotide
DPNase	=	Diphosphopyridine nucleotidase
RNA	=	Ribose nucleic acid
DNA	=	Deoxyribose nucleic acid
CoA	=	Coenzyme A

INTRODUCTION

Allison, Hoover, and Burk (1933) announced the isolation from hydrolysed yeast of a nutrilite for several strains of legume nodule bacteria. The factor was named Coenzyme R and was shown to be present in commercial sugar and in molasses. Three years later Kögl and Tonnis (1936) reported that the "bios" fractions of crystalline egg-yolk can be separated into a number of fractions, one of which is an extremely potent stimulant for the growth of yeast. The active substance was named "biotin"; it was isolated in a crystalline state as the methyl ester.

Meanwhile investigations of the nature of the toxicity of raw egg-white in higher animals, as first reported by Bateman (1916), were being undertaken. Boas (1927) demonstrated that a substance present in liver, yeast, and several other foods was able to protect rats from egg-white injury and this factor was named Vitamin H by György (1931) and "anti-egg white injury factor" by Lease and Parsons (1934).

The identity of biotin and Coenzyme R was first suggested by Nilsson *et al.* (1939) and by West and Wilson (1939), and was confirmed by György *et al.* (1940). Later in the year the same group of workers (György *et al.* (1940)) showed that biotin and Vitamin H were also identical. After this time the term "biotin" came into almost universal usage.

Intensive investigations of the chemical structure and properties of biotin

were undertaken by several groups of workers. The structure was first correctly announced by du Vigneaud (1942), while Harris *et al.* (1943) were able to confirm the structure by synthesis.

Biotin then became commercially available, and though rather expensive, was widely used in investigations of its metabolic functions. It rapidly becomes clear that, as with the other B vitamins, biotin has numerous metabolic roles in apparently unconnected processes. It was also realised that biotin is universally distributed in the biological world and is metabolically involved in all organisms so far studied.

Biotin-requiring Organisms

A very large number of organisms (see Table 1) have been shown to require an external source of biotin; indicating that most organisms either lack the ability to synthesise biotin, or are unable to synthesise sufficient to satisfy their requirements. The biotin-requirement of an organism is complicated by two factors. The first is the ability to utilise precursors of biotin, such as dethiobiotin or pimelic acid. There has been little study of these compounds with the majority of the organisms of Table 1. The second factor involves intestinal synthesis in the higher animals. For such organisms it is necessary to limit such synthesis by the use of sulfa drugs (or more recently germ-free animals), or to prevent intestinal absorption by feeding raw egg-white or avidin concentrates, or to prevent coprophagy, before the requirement can be determined.

Metabolism of Amino Acids and Proteins

Biotin has been clearly involved in the biosynthesis of a number of amino acids. The reported functions in protein biosynthesis and the conversion of some amino acids to other compounds are less certain.

The most thoroughly investigated biotin-dependent biosynthesis of an amino acid is for aspartic acid. Early investigations by Koser *et al.* (1942) established that the growth of biotin-requiring micro-organism *Torula cremoris* was increased by the addition of aspartate to a biotin-deficient medium. The biotin requirements of the organism, however, could not be completely satisfied by the amino acid. Similar studies by Stokes *et al.* (1947 a and b) established that aspartate will partially replace biotin for the growth of several bacteria.

The next stage in the investigation of the biotin-aspartate relationship was concerned with the possible dependence of aspartate deamination on biotin. Both Lichstein and Umbreit (1947 b) and Wright *et al.* (1949) established that microbial aspartate deamination was much reduced in biotin-deficiency. The deamination of other amino acids seemed also to be involved. Further detailed studies were undertaken by Lichstein and Christman (1948) who claimed that under biotin-deficient conditions the deamination of aspartate, serine, and threonine was almost absent from the four bacterial strains that they investigated. The deamination of alanine, phenylalanine, glutamate and methionine, however, appear to be unaffected. The *in vivo* activity of all the inhibited deaminases could be restored by the addition of biotin.

TABLE 1—Biotin-requiring Organisms

1. BACTERIA AND FUNGI	REFERENCES
Agrobacteria	Starr (1946)
Allescheria boydii	Villela & Cury (1949)
Ascoidea rubescens	Tanner *et al.* (1945)
Ashbya gossypii	Kögl & Fries (1937)
Bacilli	Baker *et al.* (1953), Campbell & Williams (1953), Cleverdon *et al.* (1949), Katznelson (1944), Proom & Knight (1955)
Bacterium radicicola	Nilsson *et al.* (1939)
Blastocladia prinsheimii	Cantino (1948)
Blastomyces dermatitidis	Halliday & McCoy (1955)
Botrytis cinerea	Mishra (1953)
Brucella	McCullough & Dick (1942)
Candida	Droulet & Couteau (1954), Emery *et al.* (1946), Hijner (1946), McClary (1952), Schopfer & Guilloud (1945)
Cercospora nicotianae	Steinberg (1950)
Chaetomium globosum	Buston & Basie (1948)
Clostridia	Parker (1949), Clark & Mitchell (1944), Feeney *et al.* (1943), Lamanna & Lewis (1946), Lampen & Peterson (1943), Liao (1954), Mager *et al.* (1954), Nakano & Clifton (1953), Shibata *et al.* (1949)
Collybia albicans	McClary (1952)
Cremothecium ashbyii	Schopfer (1944)
Diplodia macrospora	Margolin (1940), Stevens & Chapman (1942)
Enterococci	Niven & Sherman (1944)
Flavobacteria	Prine *et al.* (1954)
Fusarium avenaceum	Robbins & Ma (1941)
Fusarium oxysporum	Steinberg (1950)
Hansenula	Furuntani *et al.* (1953)
Histoplasma capsulatum	Salvin (1949)
Hypholoma fasciculare	Schopfer & Blumer (1942)
Lactobacilli	Oberman (1955), Rao & Sreenivasaya (1950)
Leuconostoc	Carlson & Whiteside-Carlson (1949)
Lophiodermum pinastri	Kögl & Fries (1937)
Marasmius androsaceus	Lindeberg (1941)
Memnoniella	Buston & Basie (1948), Marsh & Bollenbacher (1946)
Micrococcus sodonensis	Aaronson (1955)
Mitrula paludosa	Tanner *et al.* (1945)
Myrothecium verrucaria	Mandels (1955)
Neisseria sicca	Nemes *et al.* (1951), Ordal & Busch (1946)
Neurospora	Butler *et al.* (1941)
Ophiobolus graminis	White (1941)
Ophiostoma fagi	Tanner *et al.* (1945)
Ophiostoma piliferum	*ibid.*
Pasteurella	Berkman (1942)
Pellicularia kolergoa	Mathew (1954)
Penicillium digitatum	Wooster & Cheldelin (1945)
Piricularia oryzae	Leaver *et al.* (1947), Otani (1952, 1953), Tomizawa (1953)
Pneumococci	Bohonoa & Row (1943)
Propionibacteria	Lichstein (1955), Thompson (1943)

1. BACTERIA AND FUNGI—*ctd*	REFERENCES—*ctd*
Pythium irregulare	Steinberg (1950)
Reiter treponeme	Steinmann *et al.* (1954)
Rhizobium trifolii	West & Wilson (1939)
Rhizoctonia solani	Steinberg (1950)
Rhodospirillum rubrum	Hunter (1944)
Saccaharomyces	Leonian & Lilly (1942), Hertz (1943)
Salmonella	Lederberg (1947)
Schizosaccharomyces octosporus	Northam & Norris (1951)
Sclerotiniacamelliae	Barnett & Lilly (1948)
Sclerotium rolfsii	Steinberg (1950)
Sordaria fimicola	Lilly & Barnett (1947)
Stachybotrys	Marsh & Bollenbacher (1946)
Staphylococci	Kögl & Van Wagtendonk (1938), Gretler *et al.* (1955), Porter & Pelczar (1940)
Streptobacterium plantarum	Kuhn & Schwartz (1941)
Streptococci	Anderson & Elliker (1953), Ivanovics & Euer (1948), Niven *et al.* (1948)
Thielaviopsis basicola	Steinberg (1950)
Torula cremoris	Koser & Wright (1943)
Thichophyton album	Schopfer & Blumer (1942)
2. ALGAE AND PROTOZOA	
Amphidinium klebsii	McLaughlin & Provasoli (1957)
Amphidinium rhynchocephalum	*ibid.*
Gymnodinium breves	Wilson & Collier (1955)
Gyodinium cochnii	Provasoli & Gold (1957)
Ochromonas danica	Heinrich (1955)
Ochromonas malhamensis	Hunter *et al.* (1953)
Poteriochromonas stipatata	*ibid*
3. INSECTS	
Aëdes aegypti	Trager (1949)
Corcyra cephaloneca	Sarma (1944)
Culex molestus	Lichtenstein (1948)
Lasioderma serricorne	Fraenkel & Blewett (1943)
Ptinus tectus	*ibid.*
Stegobium paniceum	*ibid.*
Tenebrio molitor	Leclercq (1949)
Tribolium confusum	Fraenkel & Blewett (1942 a & b)
4. FISH	
Salmo fario	McLaren *et al.* (1947), Phillips & Rodgers (1950)
5. BIRDS	
Chicken	Cravens *et al.* (1944), Shaw & Phillips (1945)
Turkey	Patrick *et al.* (1941, 1943)
6. MAMMALS	
Dairy calf	Wiese *et al.* (1946), Smith (1944), Smith & Lester (1945)
Hampster	Cooperman *et al.* (1943), Rauch & Nutting (1958)
Man	Sydenstricker *et al.* (1942 a & b)
Monkey	Lease *et al.* (1937), Waisman *et al.* (1945)
Mouse	György & Poling (1940), Nielson & Black (1944)
Pig	Cunha *et al.* (1946), Lehrer (1952)
Rat	György (1931)

Contemporary with these investigations, Lardy *et al.* (1949) reported studies of the fixation of radiocarbon-labelled bicarbonate into aspartate with normal and biotin-deficient cultures of *Lactobacillus arabinosus.* The results show clearly that significant fixation of bicarbonate into aspartate occurs only in the presence of biotin. Further studies by Macleod and Lardy (1949), in which labelled bicarbonate was injected into normal and biotin-deficient rats, showed that fixation of the bicarbonate into a number of compounds, including aspartate, was much reduced in the deficient animals.

Christman and Lichstein (1950) attempted to isolate a coenzyme of the deaminases of serine, threonine and aspartate by partition paper chromatography of active extracts of *Bacterium cadaveris.* They obtained an acid-stable material that stimulated the growth of biotin-deficient yeast. Its chemical nature, however, was not established. Later studies of this material by Williams and Christman (1953) indicate that it is the coenzyme of these deaminases, but that it does not contain biotin.

Detailed studies of the interrelationship of biotin and aspartate for a number of bacterial species have been undertaken by Broquist and Snell (1951). They report that although *Lactobacillus arabinosus, L. casei,* and *Streptococcus faecalis* require increased biotin in the absence of aspartate, *L. fermenti* and *Clostridium butyricum* do not. This indicates that some micro-organisms can metabolise aspartate in the absence of biotin.

According to Nadkarni and Sreenivasan (1957) biotin-deficient rat tissues have a reduced ability to convert ethanolamine to serine.

The evidence presented and summarised above is sufficient to implicate biotin in the metabolism of aspartate, threonine, and serine. A single study by Lenti and Grillo (1953) of the splitting of threonine by *Escherichia coli* has suggested that this reaction also requires biotin.

Delwiche (1951) has reported that the particulate cysteine desulfhydrase system of an *E. coli* mutant sonic extract is activated by biotin (and also by pyridoxal phosphate, adenosine-5′-phosphate, and α-ketoglutarate). A later study by Metaxas and Delwiche (1955) of the same system in soluble alumina-ground preparations failed to show any stimulation by biotin.

Among other amino acids there is growing evidence to implicate biotin in citrulline biosynthesis. The previously mentioned studies of bicarbonate fixation by biotin-deficient rats published by MacLeod and Lardy (1949) established that arginine synthesis from bicarbonate was much reduced. Continuing this work MacLeod *et al.* (1949) established that citrulline biosynthesis was also reduced. Terroine and Rombauts (1951) studied urea formation in biotin-deficient rats and found it much less than that in the normal animal, although the reason for the reduced synthesis was not established.

It remained for the detailed investigations of Feldott and Lardy (1951) to specifically determine the role of biotin. These workers studied the conversion of ornithine to citrulline in preparations of liver homogenates from normal and biotin-deficient rats. They observed that the deficient homogenate was unable to produce citrulline from ornithine to any significant extent. In an attempt to discover the site of biotin action, L-glutamate in the first

experiment and carbamyl-L-glutamate in the second was added to the homogenates. It was reported that both the normal and deficient homogenates synthesised citrulline at the same rates with the carbamyl compound, but that only the normal homogenate can synthesise from L-glutamate. This observation implicates biotin in the synthesis of carbamyl-L-glutamate.

More recently Estes *et al.* (1956) and Sund *et al.* (1958) have studied ornithine and citrulline metabolism in biotin-deficient cells of *Streptococcus lactis* 8039. They report that such cells have a much reduced ability to convert ornithine and carbamyl-phosphate to citrulline. This group has made the claim that biotin is required for the synthesis of the enzyme catalysing this reaction. In a recent report by Ravel *et al.* (1959) it is claimed that a purified preparation of the enzyme contains only traces of biotin.

The possible sites of action for biotin in citrulline biosynthesis are outlined in Fig. 1.

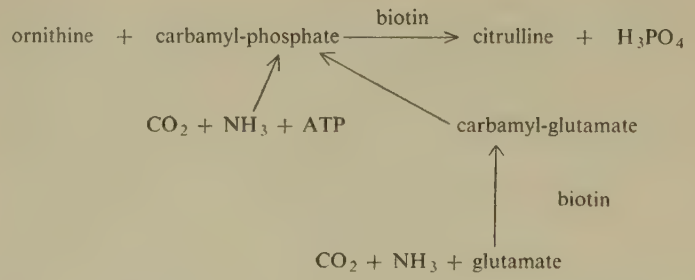


Fig. 1.—Citrulline Biosynthesis

Coon and Gurin (1949) have studied the metabolism of labelled leucine in liver tissue and determined that isovaleric acid is an important intermediate. In normal tissues this compound is converted to acetoacetate, but Fischer (1955) has reported that while mitochondria from the livers of normal rats carry out this reaction, it is almost absent with mitochondria from biotin-deficient animals. Detailed studies of the various intermediates in the conversion of isovalerate to acetoacetate have been made by Bachhawat *et al.* (1956) who have shown an important step to be conversion of β-hydroxy-isovaleryl-coenzyme A to β-hydroxy-β-methyl-glutaryl-coenzyme A. This latter compound is then split into acetoacetate and acetyl-Co A. In recent studies Woessner *et al.* (1958) have demonstrated that liver extracts from biotin-deficient rats cannot carboxylate β-hydroxyl-isovaleryl-Co A while similar extracts from normal animals can. They suggest that the enzyme catalysing the reaction is absent from the deficient tissue.

These studies show that leucine cannot be completely metabolised to acetoacetate in biotin-deficiency.

Two studies of the metabolism of methionine by biotin-deficient rats have been published. Both have been from the same laboratory and report differences in the rate of incorporation of labelled methionine into the tissue proteins of normal and biotin-deficient animals. Konikova *et al.* (1950) and Kritsman *et al.* (1953) have claimed that the rate of methionine inclusion is much reduced in the deficiency. It is unfortunate that the rates of inclusion of other amino acids into the proteins were not also determined. In the absence of any such studies it is impossible to decide whether biotin affects only the inclusion of methionine or whether all amino acids are incorporated at reduced rates. As Wagle *et al.* (1958) have reported that the rate of methionine incorporation into B_{12}-deficient liver is similarly reduced it is obviously impossible to conclude that biotin affects this process specifically unless the biotin-deficient animals were not also deficient in B_{12}. This is not clear from the Russian studies.

Investigations of the role of biotin in the metabolism of tryptophan have presented a rather confusing picture. Two different aspects have been investigated and reported, namely tryptophan oxidase and the conversion of tryptophan to niacin. These two reaction sequences cannot be regarded as independent, for niacin synthesis also involves oxidation of tryptophan; however, the end-products of the two reactions are not the same.

The earliest suggestion that tryptophan metabolism required biotin came from Meisel and Troifmova (1953) who reported that a strain of yeast being investigated for suitability for a tryptophan microbiological assay could not utilise tryptophan in the absence of biotin.

More conclusive results came from Sundaram and Sarma (1954) and Sundaram *et al.* (1953) who were studying the biosynthesis of niacin by germinating grain. They claimed that niacin synthesis was inhibited by small amounts of a biotin antagonist, ureylenecyclohexylbutyric acid. The same compound was later tested, Sundaram *et al.* (1954), on *Neurospora crassa*. Again it prevented the utilisation of tryptophan. Extending their studies to animals, Sundaram and Sarma (1955) studied the tryptophan metabolites in the urine of normal and biotin-deficient rats, and also the niacin synthesis by liver slices from each group of animals. They concluded that biotin was essential for the conversion of tryptophan to formylkynurenine. This result is apparently in opposition to the study of Dalglieshe (1955) who also studied the urinary tryptophan metabolites in deficient animals. He reported that no difference was detectable and that biotin was not involved in the conversion of tryptophan to niacin.

This difference of opinion based upon conflicting results from similar experiments obviously requires further investigations. If the Indian workers are correct then the niacin level of biotin-deficient rat liver and other tissues should be lower than with normal animals, particularly if both groups were fed diets low in niacin.

In view of this controversy, it is not clear whether biotin is concerned in tryptophan metabolism in the rat, though the studies on other organisms seem to point to biotin dependence.

In recent work Poznanskaia (1958) has investigated the adaptive formation of the tryptophan oxidase system in the livers of biotin-deficient rats.

The specific enzymatic activity of tryptophan peroxidase was measured by the decrease of added tryptophan during aerobic incubation of aqueous-saline extracts of the livers. The peroxidase activity of liver slices rose on incubation with tryptophan at about the same rate for both normal and deficient preparations. It would therefore seem that biotin is not concerned in the adaptive formation of this enzyme.

There is little evidence to show that other amino acids are affected by biotin. However the evidence is confined to single reports. For example, Baldridge and Tourtellotte (1957) have reported that the liver histidase levels of rats are significantly higher in biotin-deficiency than the normal. The synthesis of tyrosine by yeast has been claimed by Kleinzeller and Kubie (1952) to be much greater in the presence of biotin. The utilisation of glutamate may also be biotin-dependent according to Krishnaswamy and Giri (1954). Two studies of aspartic-glutamic transaminase levels of biotin-deficient rats have been reported. Rossi *et al.* (1957) claim that the transaminases are reduced by deficiency, but Baldridge and Tourtellotte (1957) were unable to detect any difference.

In regard to protein, it is still unclear whether biotin has any specific role in synthesis. Terroine and Rombauts (1953) have suggested that biotin exerts some kind of control over the rate of protein biosynthesis, while Poznanskaia (1957) has reported that the synthesis of two specific proteins, pancreatic amylase and cerum albumin, is greatly reduced in biotin-deficient chicks. Various other investigators have suggested that biotin is involved in the synthesis of specific enzyme proteins. This topic will be discussed later under the possible mode of action of biotin.

Interesting variations in the content of certain enzymes of *Neurospora* have been reported by Nason *et al.* (1951). They measured DPNase and alcohol dehydrogenase activity in normal and biotin-deficient cultures of the organism. They concluded that a marked excess of DPNase and a lack of alcohol dehydrogenase occurred in deficiency. A combined deficiency of biotin and zinc produced a striking (100 times) increase in the DPNase activity.

In summary it may be said that evidence has been presented to implicate biotin in the metabolism of aspartate, serine, threonine, citrulline, leucine, methionine, tryptophan, tyrosine, histidine, and glutamate. The evidence is not satisfactory in all cases.

METABOLISM OF NUCLEIC ACIDS

There is satisfactory evidence to implicate biotin as a necessary factor in the biosynthesis of purines. There is some evidence also which indicates that biotin may play a more general role in nucleic acid metabolism.

In the experiments of MacLeod and Lardy (1949) discussed in the previous section, a decreased synthesis of adenine and guanine from labelled bicarbonate by the biotin-deficient rat was observed. More specific information has come from investigations of biotin-deficient yeast. Moat *et al.* (1956) have shown that if excess methionine and glutamic acid are added

to the medium of deficient yeast, an aromatic amine begins to accumulate. This compound was isolated and investigated. It proved to be 5-amino-imidazole, an intermediate between formyl-glycamide and inosinic acid in purine biosynthesis. The same workers were able to show that the yeast was capable of utilising the compound on the addition of biotin to the medium. The utilisation was directly controlled by the amount of biotin.

In more recent studies, Friedman and Moat (1958) have compared the nutritional and genetic blocks in purine synthesis uring a variety of micro-organisms. They report that many biotin-deficient organisms accumulate 5-amino-imidazole riboside. Aspartic acid can replace biotin for the utilisation of this compound by biotin deficient yeast, but not for purine-requiring yeast, *Neurospora* or the vibro strains tested. It is suggested that the effect of biotin on purine synthesis may be largely through its role in aspartate synthesis.

A further observation to support the view that biotin is concerned in purine synthesis has come from the studies by Sankar and Sarma (1952) of the Indian rice-moth larvae (*Corcyra cephalonica*). It was reported that if the larvae were fed a diet containing egg-white, excretion of uric acid fell markedly. A fall in the excretion of uric acid during biotin-deficiency in rats has been reported by Ricceri (1958).

In contrast to these results are the findings of Gothoskar *et al.* (1954) who have determined the effects of biotin on the synthesis of RNA and DNA by several species of bacteria. They report that if excess biotin is added to the medium, the synthesis of both types of nucleic acid is significantly reduced. A similar, though lesser, effect is observed on the addition of aspartate or Tween 80 to the medium. At the present time there would seem to be no satisfactory explanation of this phenomenon.

The above evidence, however, is sufficient to implicate biotin at least in the synthesis of specific purines.

METABOLISM OF CARBOHYDRATES

Studies of the utilisation of sugars by biotin-deficient culture of *Leuconostoc* have been made by Carlson and Whiteside-Carlson (1949). They reported that the organism was incapable of using fructose, glucose, or invert sugar. Some use could be made of sucrose, but apparently it was metabolised only to dextran. Later these studies were extended by Moat (1954) and Moat and Lichstein (1954) to include the effects of added biotin on the fermentation of various sugars by a number of strains of yeast. In all cases biotin increased the rate of fermentation, though to varying extents with different sugars.

Contemporary with these reports Tirunarayanan and Sarma (1954a) published an account of experiments on the oxidative metabolism of various substrates, including glucose, by mycelial preparations of *Aspergillus oryzae*. Warburg techniques were used throughout. They claimed that low concentration of ureylenecyclohexylbutyric acid strongly inhibited glucose oxidation. The inhibition was reversed by the addition of biotin. The anti-biotin had no effect on the amylase activity of the mycelia.

The biotin-carbohydrate interrelationship was further demonstrated by the work of Lichstein and Boyd (1951) who reported aspartate could stimulate sugar fermentation in the absence of biotin, though its effects were less than biotin. They also measured the rates of oxidation of various sugars by yeast before and after the addition of biotin. It was claimed that not only is there an immediate marked increase, but a further lesser increase also slowly appears. From these results the authors conclude that biotin probably acts in two metabolic processes in fermentation.

Terroine (1956) has published detailed studies of the levels of various carbohydrates in the blood of biotin-deficient rats. For example, hypoglycemia and increased pyruvate appear to be typical symptoms and indicate a deranged carbohydrate metabolism. However, none of the studies discussed so far has indicated the site of action of biotin in carbohydrate metabolism.

Two papers have been published which attempt to demonstrate that biotin acts specifically in the hexokinase reaction. Williams *et al.* (1957) have studied the utilisation of various sugars, including glucose and 2-deoxyglucose by cell-free extracts of biotin-deficient yeast. Compared with the utilisation of these compounds by normal yeast it appears that only the hexokinase reaction is absent from the deficient preparations. The addition of biotin to the cell-free extracts was without effect on the fermentation.

These studies have been repeated and extended by Strauss and Moat (1958) who determined the rates of fermentation of various sugars and phosphorylated intermediates by air-dried biotin-deficient yeast. The evidence again demonstrates that hexokinase is the reaction affected in biotin-deficiency. The addition of biotin to the cells restored activity. These workers also were able to obtain a stimulation of hexokinase reactions by cell-free extracts of their organisms on the addition of biotin. However, it is interesting that although the method of preparing the extract was without effect on the hexokinase activity, biotin additions stimulated only extracts prepared by the Nossal method; they had no effect on extracts prepared by sonic vibrations.

All the above studies appear to be compatible with the hypothesis that biotin is somehow necessary for hexokinase reaction. This, however, is by far from the best studied role of biotin in carbohydrate metabolism. A great deal of attention has been paid to the role of biotin in intermediary carbohydrate metabolism in reactions involving decarboxylation or the fixation of carbon dioxide.

Lardy *et al.* (1947) have established that bicarbonate will stimulate the growth of *L. arabinosus*; the stimulation, however, occurs only in the presence of biotin. More specifically it was announced in the same year by Lichstein and Umbreit (1947 a) that the oxalacetic carboxylase enzyme of *E. coli* is absent or inactive in biotin-deficient conditions. Ochoa *et al.* (1942) reported that both this enzyme and the malic enzyme are inactive in the livers of biotin-deficient turkeys, while Shive and Rogers (1947) specifically implicated biotin in the biosynthesis of both oxalacetate and α-ketoglutarate in mammalian liver. Similar findings for the aquatic phyco-

mycete *Blastocladiella emersonii* were reported by Cantino (1953) and Cantino and Hyatt (1953).

More detailed studies of the oxalacetic reaction were made by Wessman and Werkman (1950) who used lysed preparations of *Micrococcus lysodeikticus*. They reported that in the absence of biotin there is a greatly decreased exchange of carbon between labelled bicarbonate and oxalacetate.

Studies of purified preparations of the oxalacetic carboxylase enzyme have been made by several workers. The enzyme has unfortunately not been obtained in a crystalline state, though preparations of high purity have been produced. In an attempt to determine whether biotin is an essential bound component of the active enzyme several attempts have ben made to correlate the bound-biotin content of the preparations with their activity. Plaut and Lardy (1950), Byerrum *et al.* (1950), Herbert (1950) and Vennesland *et al.* (1949) have all concluded that there is no relationship between the biotin content and the enzyme activity. However, Lichstein (1955 a, 1957) has disagreed with this conclusion and obtained apparently significant positive correlations. He claims that the failure of the previous workers to obtain his result is their insensitive assay procedures. The question, therefore, must still remain open.

A further well investigated biotin-dependent reaction is succinate decarboxylation. The necessity of biotin for this reaction was first established by Delwiche (1950) during studies of *Propionibacterium pentosaceum*. In an attempt to discover the exact function of biotin in this process Chambers and Delwiche (1954) conducted further extensive experiments but were unsuccessful. They report that biotin, although necessary for the overall reaction, did not appear to be required directly nor in the synthesis of the apoenzyme, coenzyme A or succinyl-Co A. These studies of succinate decarboxylation with *P. pentosaceum* have been repeated with similar results by Lichstein (1958) who also tested the effects of various analogues of biotin on the reactions.

Lardy and Adler (1956) have recently extended these studies to include succinate synthesis from propionate and bicarbonate by soluble enzymes from rat liver mitochondria. They report that synthesis from preparations taken from biotin-deficient animals is much reduced (see also Swick and Wood, 1960). Kaziro *et al.* (1960) have identified a biotin-coenzyme for heart propionyl carboxylase.

Several other observations have been made to show that other aspects of succinate metabolism are biotin-dependent. Olson *et al.* (1948) have compared the oxidation of succinate by slices of cardiac muscle from normal and biotin-deficient ducks. The deficient slices had a greatly reduced ability to oxidise labelled succinate and the addition of biotin *in vitro* was without effect on the reaction. However, if biotin was injected into the live deficient animal a short time before it was killed and the slices prepared, the activity of the reaction was restored almost to the normal levels.

Investigations of the same process with *E. coli* have been reported by Ajl *et al.* (1950). They established that succinate oxidation could be inhibited by the addition of small amounts of ureylenecyclohexylbutyric acid

or imidazolidine caproic acid. In both cases the activity was restored on the addition of biotin.

Miura and Iwamoto (1956) have obtained active preparations of succinic dehydrogenase from *L. casei*. They observed the enzyme to be inhibited by small amounts of both streptomycin or chlorotetracyline, but the activity could be restored by the addition of biotin.

Aside from its role in the oxalacetic-pyruvic reaction discussed previously, biotin has been suspected of being involved in other aspects of pyruvate metabolism. Pilgrim *et al.* (1942) reported that the livers of biotin-deficient rats have a much decreased ability to oxidise pyruvate, while Pilgrim and Elvehjem (1944) have made the curious observation that although magnesium ions have no significant effect on pyruvate oxidation by normal or other B-vitamin deficient livers, they further inhibit the reaction in biotin-deficient liver. Recent studies of biotin-deficient cultures of *Piricularia oryzae* by Katsuki (1955) have shown that dimethyl pyruvic acid accumulates, though no trace of this compound can be found in normal cultures. The significance of this observation is still obscure, though Katsuki (1959a) has shown that there is no significant reduction in Coenzyme A biosynthesis by the deficient fungus as might have been expected from the above result. In a further study (Katsuki, 1959b) has observed accumulation of large amounts of alpha-keto acids in the medium of biotin-deficient *Bacillus macerans,* accompanied by a decrease in coenzymes (including Co A) and an increase in DPNase.

The evidence presented above illustrates that biotin is involved in carbohydrate metabolism at several levels. The specific reactions which appear to be biotin dependent are hexokinases, oxalacetic carboxylase, succinic carboxylase, α-ketoglutarate synthesis, succinic dehydrogenase and pyruvate oxidation.

METABOLISM OF LIPIDS

Early studies of lactic acid bacteria by Williams *et al.* (1947) established that in biotin-deficient conditions, oleic acid becomes a necessary growth factor. Broquist and Snell (1948) also claimed that the growth stimulation by oleic acid was absent in the presence of avidin. This stimulation of a micro-organism by oleic acid in the absence of biotin has been reported for several organisms including *Neurospora crassa,* by Hodson (1949).

Detailed studies of the biotin-like activity of oleic and other fatty acids were carried out by Cheng *et al.* (1951) and O'Leary (1959) who showed that the amount of activity was dependent upon both the degree of unsaturation and the position of the double bond in the molecule.

Other investigations of the effects of avidin on the stimulant effect of oleic acid have been carried out by Broquist and Snell (1951) who revised their earlier findings and conclude that *L. plantarum* is the only species affected by avidin. Traub and Lichstein (1956) have also carried out investigations of this organism and their results indicate that oleic acid affects the permeability of the cells to biotin. It may therefore be that biotin is not concerned in the biosynthesis of oleic acid in these micro-

organisms as had been suggested, but that when added to a biotin-deficient medium, oleic acid allows those traces of biotin which are present to enter the cells.

A further fatty acid-biotin relationship has become apparent recently from the studies of Wakil *et al.* (1958) on the biosynthesis of long chain fatty acid by avian liver. They have established that the synthesis of such compounds probably occurs by a process which is not the reverse of the well known β-oxidation pathway. They have also prepared a number of enzyme fractions from liver which catalyse this synthesis, and have shown that avidin inhibits the incorporation of bicarbonate into the fatty acid intermediates. The biotin-dependent reaction appears to be the formation of malonyl-CoA from acetyl-CoA (see Stumpf, 1960, for details).

Biotin has been implicated in the metabolism of a number of other lipids. For example, Gavin and McHenry (1941) studied the effects of B-vitamin administration on lipid metabolism and concluded that biotin administration causes fatty livers with high cholesterol levels. This condition was prevented by feeding egg-white. Okey (1946) later reported that guinea pigs fed only two micrograms of biotin daily had liver cholesterol levels twice those of deficient animals.

In contrast to these findings Curran has reported (1950) studies of cholesterol biosynthesis in biotin-deficient rats using heavy water. It was concluded that biotin was without effect on cholesterol synthesis though fatty acid synthesis was slightly affected. This result is difficult to accept for the earlier findings have been repeated by two groups of workers. Arrigo and Malagomba (1952), studying the relationships of prothrombin and cholesterol, were able to duplicate the earlier studies of Okey (1946), while Gram and Okey (1958) have also confirmed the earlier work and shown that the synthesis of cholesterol, phospholipids, and glycerides from labelled acetate by biotin-deficient rats is much reduced.

It would therefore seem that biotin is involved in the metabolism of a number of lipids. The evidence indicates some relationship between biotin and unsaturated fatty acids for some micro-organisms, though this may only be one of cell permeability. Biotin also appears to be necessary for the synthesis of long chain fatty acids and cholesterol in higher animals.

VITAMIN INTERRELATIONS

Evidence has been presented to implicate biotin in the synthesis, mode of action, and general metabolism of a surprising number of other vitamins and growth factors. In most cases, however, the evidence is not completely satisfactory to establish a definite biotin-dependence.

The most interesting vitamin interrelationships of biotin are with other B-vitamins. One of these has already been discussed in the previous section on amino acid metabolism where the evidence for a possible role of biotin in the biosynthesis of niacin was outlined. Other observations by Rose and Nickerson (1956) have indicated a relationship between biotin requirements and the retention of niacin by eight strains of yeast. In later experiments

Rose (1960a) has shown that biotin controls the utilisation of nicotinic acid compounds that are intermediates in pyridine nucleotide biosynthesis. It is suggested (Rose 1960b) that the major effect may concern the requirement of biotin for adenine biosynthesis (the purine moiety of the nucleotides) for the addition of adenine to the biotin-deficient yeast suppresses the secretion of nicotinic acid compounds.

A relationship between biotin and riboflavin is also possible following the studies of Tirunarayanan and Sarma (1954b). These workers observed that gammexane will inhibit *Aspergillus oryzae* and that riboflavin synthesis is particularly depressed. Inositol is without effect on the inhibition, while folic acid increases it. Biotin, however, is able to restore riboflavin synthesis almost to normal, though only in the absence of folic acid. More definite results came from a later study by Tirunarayanan *et al.* (1954) who found that riboflavin synthesis in the same organism can be strongly inhibited by either ureylenecyclohexylbutyric acid or biotin sulfone. In both cases the synthesis was restored on the addition of biotin. The explanation of these effects may not be as direct as these workers suggest. Johnson (1955) has shown that the precursors of riboflavin are purine bases and it is known that purine synthesis is inhibited in the absence of biotin.

The above work also illustrates a possible relationship between biotin and inositol. This is supported by the studies of Tirunarayanan and Sarma (1953) with *N. crassa*, which is also inhibited by gammexane, though one part of biotin will overcome an inhibition of growth caused by 10,000 parts of gammexane. In the normal organism inositol cannot overcome the inhibition, but in an inositol-less mutant species it can. The workers also report that biotin is without effect on the inhibition in the normal organism if the supply of inositol is limited.

Aside from the antagonism between biotin and folic acid in the gammexane inhibition studies mentioned above, there is other evidence of a relationship between folic acid and biotin. For example, Mitbander and Sreenivasan (1954) have reported that the synthesis of a compound with folic acid activity by *L. arabinosus* is markedly increased on the addition of biotin to the medium. Recent studies by Luckey *et al.* (1955) of the metabolism of germ-free rats have also reported a similar phenomenon. They report that the germ-free animals, unlike the normal, require a dietary source of biotin. Measurements of folic acid excretion have shown that it is greatly depressed in biotin-deficiency and is restored on the addition of biotin to the diet.

Of the remaining B-vitamins there is some evidence to implicate thiamine synthesis as biotin-dependent. Fukui and Kishibe (1951) have reported that the synthesis of bacterial thiamine by several species is greatly increased on the addition of biotin. Also of interest are the observations of Ikehata (1956) who has reported that the production of the thiaminase of *Bacillus aneurinolyticus* is biotin-dependent.

A relationship between biotin and vitamin C has been suggested by De Felice (1950, 1952a) who claims that the two compounds are synergistic to each other. In later studies De Felice (1952b) has published detailed analyses of various tissues of guinea pigs on normal and scorbutic diets,

with and without added biotin. His results indicate that biotin may spare the requirements of ascorbic acid by some tissues. De Felice (1954) has concluded that biotin assists in the preservation of vitamin C in the tissues of the guinea pig. This result is the direct opposite of that of Terroine (1954) who has reported that ascorbic acid, as well as methylene blue or neutral red, affords considerable protection to the rat on an egg-white diet from the symptoms of biotin-deficiency. Similar findings for *L. arabinosus* have been reported (Terroine, 1958).

The evidence presented in this section indicates an interrelationship between biotin and the following vitamins: niacin, riboflavin, inositol, folic acid, thiamine, and vitamin C.

OTHER METABOLIC FUNCTIONS

A number of publications have presented evidence to implicate biotin in a variety of metabolic processes that are not readily classified into the preceding sections. These reports will therefore be considered separately.

Appleby *et al.* (1947) have suggested that the synthesis of tyrothricin by *Bacillus brevis* may be biotin-dependent. Their results show that the synthesis of tyrothricin by submerged cultures of the organism is stimulated by the addition of biotin to the medium.

During studies of a bacterial formic hydrogenase system Lichstein and Boyd (1951) reported that maximum activity was observed only in the presence of biotin or oleic acid. Similarly Ericson and Harper (1955) have claimed that the betaine-homocysteine transmethylase of rat liver is greatly depressed in biotin-deficiency. Porphyrin synthesis by *Rhodopseudomonas spheroides* is also reduced in the absence of biotin.

Inhibitions of some metabolic processes in the presence of excess biotin has been reported by some workers. Wiken and Ghose (1954) have claimed that although sulfate reduction by *Desulfovibrio desulfuricans* requires the presence of biotin, excess is inhibitory. Similarly Elizarov and Meisel (1949) have reported that the catalase activity of a number of micro-organisms is reduced in the presence of excess biotin.

For the most part, however, the reactions discussed in this section have been confined to single studies and it would be unwise to draw any definite conclusions until more detailed information is available.

MODE OF ACTION

The evidence presented in the previous sections establishes biotin to be an essential factor for many metabolic processes. In an attempt to establish the mode of action of biotin in these processes it is necessary to consider the chemical changes which are alleged to be biotin-dependent. These are outlined in Table 2. Only those reactions for which there is good evidence for the participation of biotin are included.

A detailed consideration of these reactions has been undertaken by several workers in an attempt to find some underlying chemical process that is common to all biotin-dependent reactions. Thus far it cannot be said that any such process has yet been demonstrated, but a number of interesting hypotheses have been proposed. These will be considered briefly.

TABLE 2—BIOTIN-DEPENDENT REACTIONS

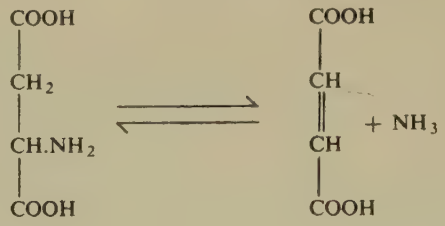
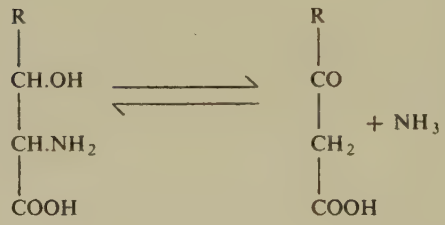
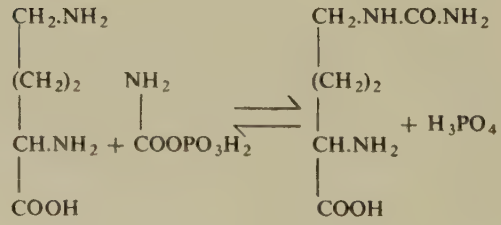
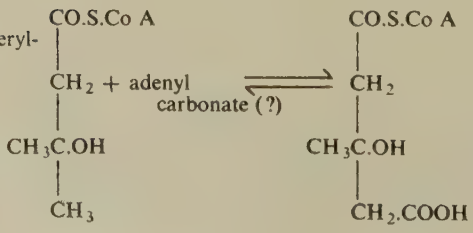
Reaction	*Probable Chemical Changes*
1. aspartate deamination	
2 serine or threonine deamination	
3. citrulline biosynthesis	
4. carboxylation of β-hydroxy-isovaleryl-Co A	

TABLE 2—*continued*

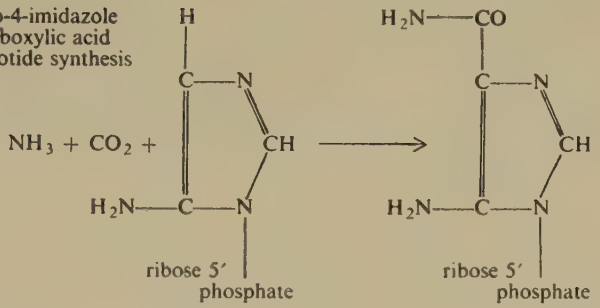
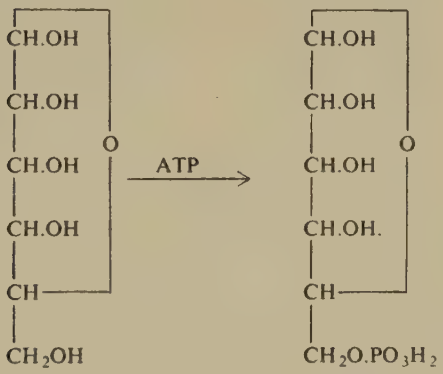
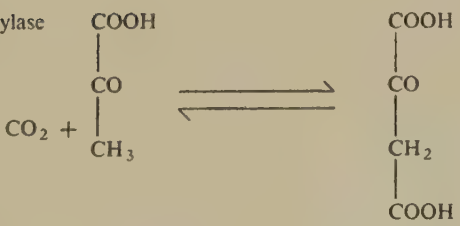
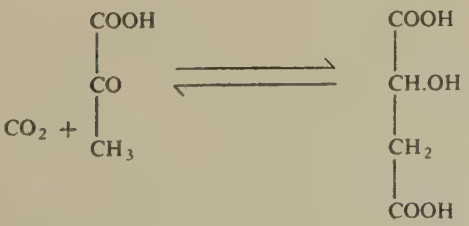
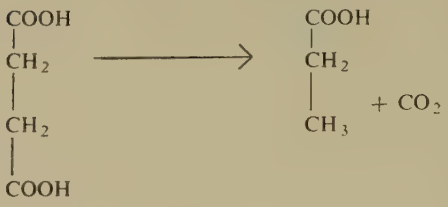
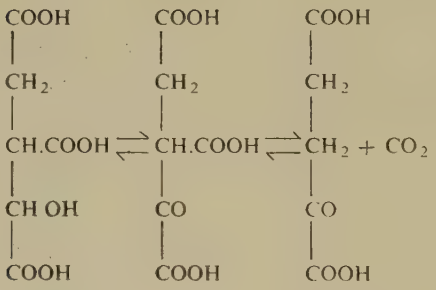
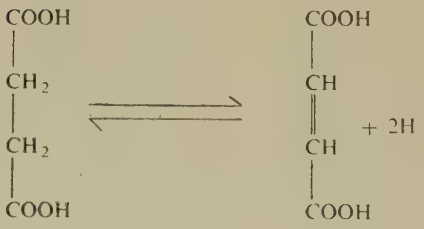
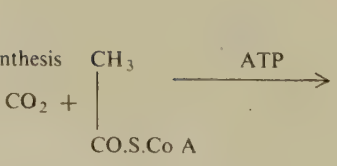
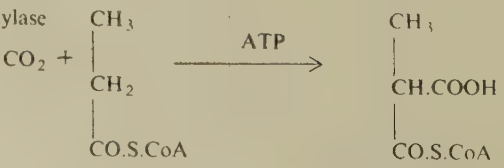
Reaction	*Probable Chemical Changes*
5. 5-amino-4-imidazole carboxylic acid ribotide synthesis	
6. hexokinase	
7. oxalacetic carboxylase	
8. malic enzyme	

TABLE 2—*continued*

Reaction	*Probable Chemical Changes*
9. succinic decarboxylase	
10. α-ketoglutarate synthesis	
11. succinic dehydrogenase	
12. malonyl-Co A synthesis	
13. propionyl carboxylase	

It is obvious that many of the above reactions involve the fixation or removal of carbon dioxide from a molecule. At least six of the twelve reactions given fall into this category and others in which the exact site of biotin action is undiscovered may also be of the same type. Three hypotheses have been proposed to account for the role of biotin in carboxylations and decarboxylations.

The first suggestion is by Melville *et al.* (1949) who proposed that the biotin molecule may be unstable during enzymic changes and may be capable of losing the carbonyl group of the imidazole ring in the manner shown in Fig. 2. To test this hypothesis the workers prepared biotin by a chemical

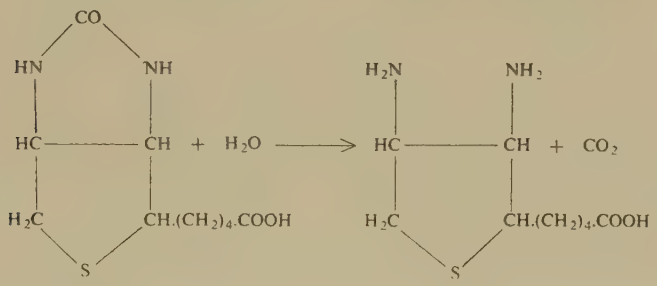


Fig. 2—Hypothetical Decarbonylation of Biotin

synthesis that allowed them to introduce ^{14}C-labelling into the carbonyl group. This compound then was studied for stability during a carboxylation reaction and it was reported that no exchange of ^{14}C between the biotin and the substrate could be detected. It therefore must conclude that carboxylation reactions do not involve the removal of this part of the biotin molecule.

The second hypothesis of the mode of action of biotin is that an unstable biotin-carbon dioxide complex is involved in the reaction. This complex can be formed during decarboxylations, or decomposed during carboxylations. Recent reports (Lynen *et al.*, 1959a; Lynen 1959; Wakil and Gibson, 1960) have produced evidence for the existence of this complex during reactions catalysed by acetyl- and beta-methylcrotonyl Co A carboxylases. The studies have been extended by Kaziro *et al.* (1960) to the propionyl carboxylase of pig heart and the evidence for a biotin coenzyme in these systems is now very strong. The proposed mechanism (Calvin and Pon, 1959) is given in Figs 3 and 4. The inhibitions by avidin of carboxylation systems (Wakil *et al.*, 1958; Utter and Keech, 1960; Swick and Wood, 1960; Fletcher and Myant, 1960) are strong supporting evidence.

However, it is difficult to see how this hypothesis can explain those metabolic functions of biotin which do not involve carbon dioxide fixation and removal. It also remains to be explained why many biotin-dependent reactions are not stimulated *in vitro* on the addition of biotin, and why many enzymes catalysing such reactions contain only traces of biotin.

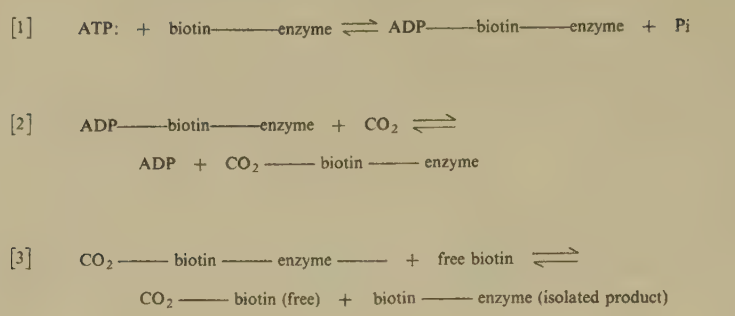


Fig. 3—Lynen's Hypothesis

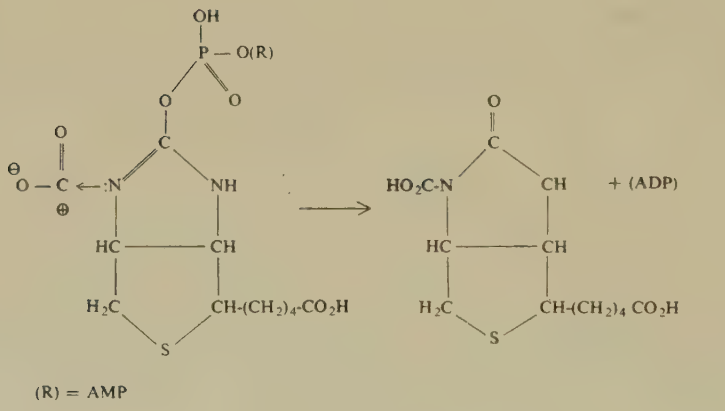


Fig. 4—Structures of Hypothetical Intermediates

A more general hypothesis of the mode of action of biotin has come from Lichstein (1951) who has suggested that all biotin-dependent reactions involve either inter- or intra-molecular hydrogen exchange. For example, most decarboxylations can be regarded as processes such as that depicted in Fig. 5, while deaminations also involve the removal of a hydrogen atom. The same is true of the hexokinase reaction. Lichstein's proposal is illustrated in the scheme given in Fig. 6.

There is no experimental evidence to confirm this hypothesis, though Traub (1959) has recently made some interesting suggestions on this topic. From detailed X-ray crystallographic analysis of crystalline biotin it is suggested that the hydrogen atom of the hydroxyl group of the valeric acid side chain may be capable of forming a hydrogen bond with the oxygen atom of the carbonyl group of the imidazole ring. This bonding may even occur in solutions of biotin. Such an intra-molecular type of bonding would tend to alter the charge distribution of the imidazole ring in such a way as

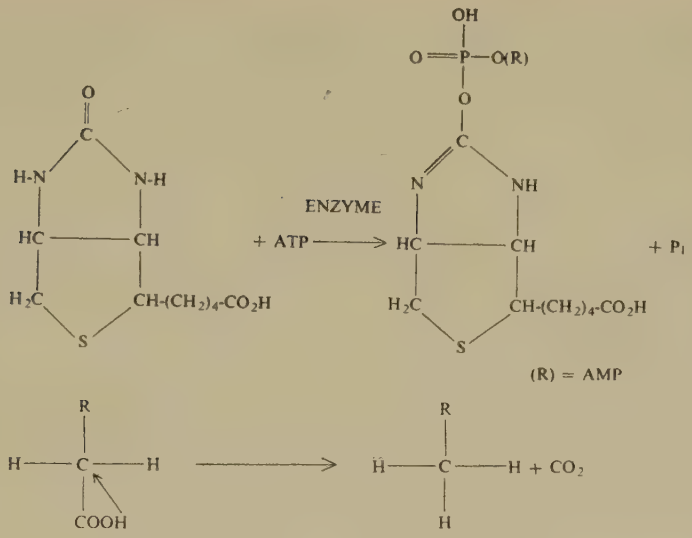


Fig. 5—Intramolecular Hydrogen Exchange

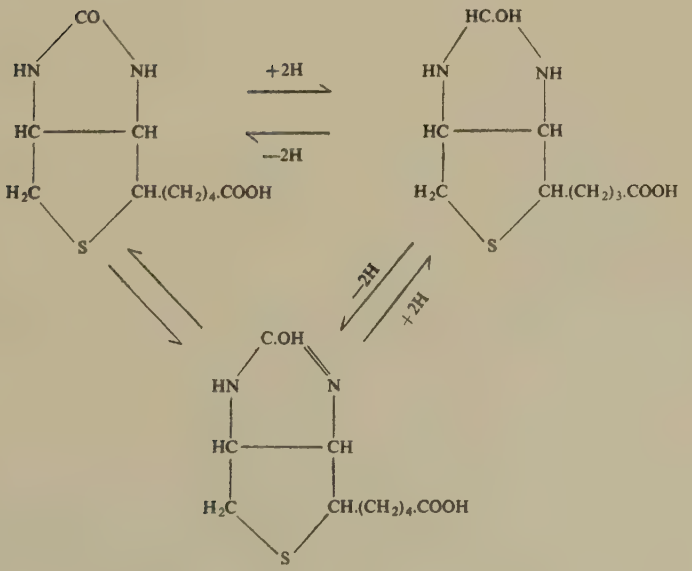


Fig. 6—Lichstein's Hypothesis

to favour the enol structure of the keto-enol equilibrium. This in turn would enhance the reactivity of the nitrogen atoms and perhaps enable the acceptance of a proton as required by Lichstein's hypothesis, or even of ADP as required by Lynen (1959). The known biological properties of structural analogues of biotin in which this hydrogen bonding is affected also supports this hypothesis.

Unfortunately, even though Traub's results can be regarded as favouring the hypothesis that biotin functions in a biochemical transport scheme, there is no definite evidence one way or the other for most biotin-dependent systems. In fact all hypotheses which postulate a direct coenzyme function for biotin in enzymatic processes must explain the previously discussed failure of biotin to stimulate *in vitro* systems. The failure of most workers (Briggs 1960; Ochoa *et al.* 1942; Tietz and Ochoa, 1959; Ravel, *et al.*, 1959; Hamilton and Westheimer, 1959) to find bound-biotin in purified enzymes, known to catalyse biotin-dependent reactions, can be regarded as evidence against the coenzyme hypotheses for the enzymes studied. However, as Lichstein (1949a, b) has pointed out, biotin itself may not be the active coenzyme; it may be only a part of a larger structure which may be undetectable by the usual assay methods. There is no evidence for the existence of any such compound at the present time.

In the face of these difficulties to a general coenzyme hypothesis, the attention of some workers has been directed to the hypothesis that biotin functions somehow in the synthesis of enzymes. This hypothesis has the great advantage of explaining the failure of biotin to stimulate purified systems. The findings of Poznanskaia (1957) that the synthesis of certain specific proteins is much reduced in biotin-deficient chicks and the results of Konikova *et al.* (1950) and Kritsman *et al.* (1953) indicating that the incorporation of methionine into tissue proteins is reduced in the absence of biotin, can both be regarded as evidence in favour of the enzyme synthesis hypothesis. However, such evidence is not conclusive as protein synthesis is reduced in many types of deficiency. It would nevertheless be of interest to determine the methionine contents of enzymes known to catalyse biotin-dependent reactions.

More satisfactory evidence for the hypothesis has come from studies of the ornithine-carbamyl phosphate reaction by Sund *et al.* (1958). It was found that biotin-deficient cells of *S. lactis* had a greatly diminished ability to convert ornithine and carbamyl phosphate to citrulline. Normal activity was restored on the addition of biotin, glucose and an animo acid supplement containing glutamine. A period of incubation was also necessary. It was found that although uracil is not required to restore activity, the addition of small amounts of 5-fluorouracil completely blocks the restoration of activity; this effect can be overcome by adding uracil. It was also discovered that the addition of analogues of other compounds probably involved in protein synthesis inhibit the restoration. The analogues used were 8-azaguanine, 4-oxa-DL-lysine, and β-(2-thienyl)-DL-alanine. Again the inhibitory effects could be overcome by the additions of the normal compounds, e. e. guanine, lysine and alanine respectively. The weight increase of the cells incubated in the complete normal medium was 14% compared with an increase of 12% in the absence of biotin. Considering these results the

workers conclude "that synthesis of the ornithine-citrulline enzyme is not merely a result of increased cellular material and that biotin plays a specific role in the synthesis of the enzyme".

Similarly, Plaut (1961) has shown that although biotin nutrition appears to be without effect on malic enzyme biosynthesis by *L. arabinosus*, the known ability of the organism to manufacture small amounts of biotin (Broquist and Snell, 1951) may be adequate to fulfill the needed function in the formation of active malic enzyme.

Encouraging as these results appear to be for the enzyme-synthesis hypothesis it must be remembered that other workers who have attempted to show that biotin is concerned in the synthesis of enzymes for specific reactions have reached negative conclusions. An excellent example of such results are the findings of Chambers and Delwiche (1954) with the succinic decarboxylase system. Similarly during studies of the hexokinase reaction, Briggs (1959) obtained an inhibition of glucose fermentation by air-dried, biotin-deficient yeast with both norbiotin and homobiotin. The fermentation was stimulated by the addition of crystalline hexokinase, and this stimulation was enhanced by biotin, though decreased by the biotin homologues. No inhibition or stimulation was obtained, however, when the compounds were tested on an *in vitro* system. This evidence indicates that biotin is not concerned in the synthesis of the hexokinase enzyme, though as the purified enzyme contained only traces of bound biotin, the function of the vitamin in the reaction is obscure.

Other evidence against the enzyme-synthesis hypothesis has been presented by Wessman *et al.* (1954). They observed that although biotin-deficient cells of *Micrococcus lysodeikticus* cannot fix carbon dioxide into oxalacetate, the reverse reaction is unaffected; indicating that the oxalacetic carboxylase enzyme is present in biotin-deficiency. Similarly the inhibitions of biotin-dependent CO_2 fixation systems by avidin (Wakil *et al.*, 1958; Utter and Keech, 1960; Swick and Wood, 1960; Fletcher and Myant, 1960; Kaziro *et al.*, 1960) appear incompatible with this hypothesis for these carboxylases.

It is therefore impossible to draw any definite general conclusion about the role of biotin in enzyme reactions. Although no hypothesis of mode of action is completely satisfactory for all systems, the evidence indicates that biotin is involved as a coenzyme in some carboxylation reactions. The hypothesis that biotin is concerned in enzyme biosynthesis in other systems cannot be dismissed. That biotin plays two distinct and different functions in enzyme reactions remains the most likely alternative.

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REMOVAL OF FLUORIDE FROM FLUORIDATED WATER CONTAINING 1 p.p.m. FLUORIDE

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Summary

The fluoride ion can be completely removed from water by simple treatment with commercial bone flour, or preferably, calcined bone flour. One gramme of bone flour will retain approximately 1 mg fluoride at pressures up to 40 lb/sq. in. and at flow rates equivalent to 70 gal/sq./ft./min.

INTRODUCTION

The Report of the Commission of Inquiry into the Fluoridation of Public Water Supplies (1957) refers to the subject of defluoridation and states that an efficient filter could be produced in New Zealand if it were needed, for household purposes. The following work was undertaken to test the efficacy of a simple defluoridation process which would enable anyone who so desires to remove fluoride from water fluoridated at 1 p.p.m., at low cost.

Methods of removing fluoride ion from water have been reviewed by Smith (1939) and Maier (1947, 1953). A wide range of adsorbents have been tested, and large-scale plants are in successful operation in the United States and elsewhere (Maier, *op. cit.*, Forrest *et al.*, 1953, Cillie, 1958). Small scale filters for domestic use have also been described (Smith, Forrest *et al.*, *op cit.*). This paper describes some experiments using bone flour and calcined bone flour to remove fluoride from water.

EXPERIMENTAL

The bone flour was commercially available material used in animal feeds. Different samples, of varying quality, have given equally good results, although some required more preliminary treatment than others, chiefly because of extraneous matter. One sample could be used after simply washing to remove fines, but it was usually necessary to boil the bone flour in normal alkali for a short time before washing. Calcined bone flour was prepared by putting the material (as purchased, if clean) in a muffle furnace at 600°C for 10 min. The charred bone flour was then wet-sieved and dried.

Acid treatment of calcined bone flour as described by Smith (1939) did not effectively increase fluoride capacity of the product.

To achieve high capacity at reasonable rates of flow, a particle range of 40–100 mesh was chosen for the columns to be described, although coarser

and finer grades were also effective. Columns were prepared by allowing the material to settle by gravity from a slurry in water. Both distilled and tap waters were used in the early stages, but as these gave identical results, tap water only was used thereafter. Water from a chlorinated supply gave the same results as untreated water. A stock solution of reagent-grade sodium fluoride was used to fluoridate small quantities of water as required. Concentrations greater than 1 p.p.m. F′ were sometimes used in order to test the ability of bone-flour columns to deal with such concentrations and to save time.

Fluoride estimations were made by the thorium-nitrate-alizarin back-titration procedure, described in detail by Harrison (1949). Column effluents could be analysed directly only when the bone flour had been previously boiled in several lots of alkali, otherwise phosphate, and other interfering substances gave rise to misleadingly high results. After being made alkaline, therefore, samples were concentrated in platinum basins and subjected to steam distillation from perchloric acid.

RESULTS

Capacity of Bone Flour Column

Thirty grammes of bone flour formed a column ½ in. diameter by 6 in. high, with a flow rate of 3 ml per min under a head of 12 in. of water. Two litres of water of 1 p.p.m. F′ content were passed through, followed by 2 litres of 5 p.p.m. F′, followed by 2 litres of 10 p.p.m. F′. The fluoride content of the final portions of effluent was 0·08, 0·35, 0·5 p.p.m respectively, so that after absorbing 32 mg F′ the column was still retaining 95% of the applied fluoride. A similar column retained 31 mg F′ from water containing 10 p.p.m. F′ before the fluoride content of the effluent rose to 1 p.p.m. On eluting with 1 litre of N NaOH, 30·2 mg F′ were recovered. After washing, the column readily retained a further 20 mg F′ applied at 1 p.p.m. level.

Calcined Bone Flour

Bone flour is subject to algal growth and decomposition if kept for any length of time in a moist state, but calcined bone flour will remain in water for at least one week without deterioration. It was used for the experiments with larger columns. A column 2 in. diameter by 5·5 in. high, containing 220 g calcined bone flour was used to test the effect of increasing flow rates, applying pressure with the aid of a cylinder of nitrogen. The pressure was increased in steps of 5 lb/sq. in. from atmospheric to 25 lb/sq. in. At each pressure 450 ml of water containing 2 p.p.m. F′ were passed through, of which the final 100 ml was collected for analysis. The column outflow was equivalent to 1 gal in 35 min at atmospheric pressure increasing to a rate of 1 gal in 1·6 min at the maximum pressure. This is equivalent to a flow rate of 27·5 gal/sq. ft per min, but even at this flow, no fluoride was detectable in the effluent. A further 31·5 litres (2 p.p.m. F′) were passed through at 15 lb/sq. in., before this column became clogged by suspended material in the water. At this point there was still no fluoride appearing in the effluent.

A domestic high pressure water filter, originally designed for earthenware filter candles was adapted by replacing the candle with a length of 1½ in. diameter polythene tubing, closed at the lower end by a circle of glass fibre cloth, heat-sealed directly to the tube. In this 97 g of calcined bone flour, 40–60 mesh, made a column 5 in. high, topped by a 1 in. layer of acid-washed sand. A small inverted polythene funnel resting on the sand had its neck inserted into the bung closing the tube; this acted as a collector, enabling the whole upper surface to be used. Water containing 1 p.p.m. F′ was passed through this apparatus at a pressure of 40 lb/sq. in., the outflow rate being 0·84 gal/min (72 gal/sq. ft/min). After 64 litres had passed, no fluoride could be found in the effluent, but after this the level rose gradually until it reached 0·25 p.p.m. at 107 litres.

The strong affinity of bone flour for fluoride was well demonstrated by the following test. Two grammes of calcined bone flour was shaken in 200 ml of water containing 1 p.p.m. F′, for 3 min. A sample taken after 15 min settling contained 0·12 p.p.m. F′, but after further standing overnight, the fluoride level had fallen to zero. This experiment was repeated several times; it shows that small quantities of water could easily be defluoridated without any special apparatus.

DISCUSSION

Calcined bone flour used in these experiments did appear to cause a slight change in the taste of the water, but this was not at all objectionable. Although calcined bone flour is said to be expensive for large-scale use (Maier, 1953), it should be very useful for domestic use, since the low initial cost (4d. per lb) would make regeneration unnecessary. Bone flour produced in New Zealand appears to be quite suitable after calcining at 600°C for 10 min followed by wet-sieving. It would no doubt be feasible to make a small de-mountable filter of polythene to be attached directly to a tap, and to recharge this with fresh material each two to three weeks.

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PELAGIC HYPERIIDEA (CRUSTACEA : AMPHIPODA) COLLECTED BY THE *MAGGA DAN* BETWEEN AUSTRALIA AND ANTARCTICA WITH SOME NOTES ON THEIR DISTRIBUTION

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Summary

A small collection of Amphipoda Hyperiidea collected by the Australian National Antarctic Research Expedition during a voyage of the *Magga Dan* between Australia and Antarctica early in 1959 is described. The collection lends support to theories on the distribution of certain common species.

Introduction

The material described below was collected in the summer of 1958–59 by Mr Norman Dyson of CSIRO, aboard the A.N.A.R.E. ship *Magga Dan* on a voyage to and from Antarctica, by Clarke-Bumpus nets and Hardy Plankton Indicators. Although the collection is small it gives a useful cross-section through different water masses and illustrates changes in predominant plankton species. It also allows some comment on points of systematic interest. Examination at the same time of other CSIRO material from Bass Strait and of Stanford University Antarctic Collections has assisted in clarifying some of these points.

Station List

Sta. 2. 42° 15′ S, 141° 47′ E. Surface. 8/1/59. 0410–0510 hr. Hardy Indicator.

Sta. 3. 42° 46′ S, 141° 25′ E. Surface. 8/1/59. 0800–0900 hr. Hardy Indicator.

Sta. 5. 43° 48′ S, 140° 40′ E. Surface. 8/1/59. 1600–1800 hr. Hardy Indicator.

Sta. 23. 56° 13′ S, 138° 14′ E. 100m–0m. 11/1/59. 1545–1600 hr. Clarke-Bumpus.

Sta. 29. 60° 35′ S, 136° 00′ E. 100m–0m. 12/1/59. 1545–1600 hr. Clarke-Bumpus.

Sta. 57. 64° 43′ S, 148° 54′ E. 100m–0m. 11/2/59. 1545–1600 hr. Clarke-Bumpus.

Sta. 60–63. 65° S, 147–152° E. Surface. 16/2/59. 0815–2115 hr. Hardy Indicator.

Sta. 64–65. 64° 28′ S, 154–157° E. Surface. 17/2/59. 0000–0300 hr. Hardy Indicator.

Sta. 76. 61° 42′ S, 158° 25′ E. 100 m–0m. 25/2/59. 1545–1600 hr. Clarke-Bumpus.

Sta. 77. 60° 41′ S, 158° 55′ E. Surface. 25/2/59. 2000–2100 hr. Hardy Indicator.

Sta. 81–82. 57° 38′ S, 158° 54′ E. Surface. 26/2/59. 1600–2000 hr. Hardy Indicator.

Sta. 88. 57° 35′ S, 156° E. 100m–0m. 28/2/59. 1545–1558 hr. Clarke-Bumpus.

Sta. 90. 50° 32′ S, 154° 42′ E. Surface. 28/2/59. Hardy Indicator.

Sta. 91. 49° 22′ S, 153° 25′ E. Surface. 1/3/59. 0500–0900 hr. Hardy Indicator.

Sta. 92. 48° 52′ S, 152° 43′ E. Surface. 1/3/59. 1200–2200 hr. Hardy Indicator.

SYSTEMATICS

AMPHIPODA HYPERIIDEA

Family VIBILIIDAE

Cyllopus magellanicus Dana, 1853.

Hurley, 1955 : 129, figs 23–50; Hurley, 1960 : 111.

OCCURRENCE: Sta. 76, 1 female, 10 mm.

Family HYPERIIDAE

Hyperia cf. *Hyperia agilis* Dana, 1852.

Dana, 1852, p. 986–987, pl. 67, fig. 11 a–d.

Bovallius, 1889: 195–196, fig. 1.

OCCURRENCE: Sta. 5, 1 juvenile or female, 3½ mm.

REMARKS: I have been unable to place this specimen in any of the commoner species of *Hyperia*. Superficially it is not unlike *H. macrodactyla* Stephensen (Stephensen, 1924, pp. 90–91; Yang, 1960, pp. 35–38, fig. 9) in having relatively prominent spination on the anterior margins of segments 4–6 of peraeopods 3–5. The dactylos of each peraeopod is also long. However, it differs from *H. macrodactyla* in having Pr. 3 as long as Pr. 4, both being longer than Pr. 5. All three epimeral plates are distinctly right-angled posterodistally, and the outer ramus of each of the uropods is distinctly shorter than the inner. Few species of *Hyperia* have distinct spination on these last three peraeopods on segments 5 and 6 and the only species in the literature which appears similar is that described by J. D. Dana in 1852 as *Hyperia agilis*, one which has not, to my knowledge, been reported since, although Bovallius believed that it might be 'a good species'.

Dana's figures and description are not over-generous but show that all three epimeral plates are right-angled posterodistally and that peraeopods 3–5 have spines on segments 4–6. However, his figure 11a indicates some spination on the posterior as well as the anterior margins. It is also obvious from Dana's figures that Pr. 3 and 4 in *H. agilis* are subequal, the 5th slightly shorter. This aside, having been led to *H. agilis* on morphological grounds, it is of particular interest to find that Dana's specimens were taken in the Pacific – "Lat. 41° South; Long. 76° 25′ West. Collected several specimens, April 5, 1839 some of which were in the water-cavity of Salpas; also between New Zealand and New Holland". The A.N.A.R.E. specimen was taken at 43° 48′ S, 140° 40′ E.

While hesitating to announce the definite re-discovery of Dana's species on the evidence of one slightly battered specimen, the author is inclined to consider that this specimen probably belongs to Dana's species.

Parathemisto gaudichaudii (Guerin), 1825.

Hurley, 1955 : 161, figs 159–174; Bowman, 1960 : 379–382, figs 16a–17.

OCCURRENCE: Sta. 23, 1 ovigerous female, 10½ mm ('short-legged'); Sta. 29, 1 female, 10½ mm (short-legged); Sta. 57, 1 female, 11½ mm (short-legged but carpus of Pr. 1 and 2 distinctly expanded); Sta. 60–63, 1 female, 10 mm; Sta. 77, 1 female, 12 mm (var. *thomsoni,* cf. pl. 174, Stebbing, 1888); Sta. 81–82, 3 females, 3–4 mm (cf. pl. 173, Stebbing); Sta. 88, 14 juveniles, 2½ mm; Sta. 90, 3 females, 3½–5 mm (cf. pl. 173); Sta. 91, 1 female, 7 mm and fragments of 2 more specimens (cf. pl. 173); Sta. 92, 5 females, 3–10½ mm, 4 males, 10½ mm.

REMARKS: The differences between the "varieties" of this species have been commented on elsewhere (Hurley, 1960 : 112).

Parathemisto gracilipes (Norman), 1869.

Hurley, 1955 : 153–161, figs 133–158, 176, 178; Bowman, 1960 : 375–379, figs 11, a–i; 14–15; 16, b.

OCCURRENCE: Sta. 2, 4 males, 6½–7½ mm. 10 females, 4–8 mm (1 with well-developed broodplates); Sta. 3, 2 males, 6½–7 mm. 2 females, 5 mm; Sta. 92, 1 female, 5½ mm.

The serration of the inner ramus of uropod 3 (see key in Bowman, 1960) adequately separates *P. gaudichaudii* from *P. gracilipes.* The inner margin in *P. gaudichaudii* is completely without serration. However, some further comments on the distinction between *P. gracilipes* and *P. australis* (see Hurley, 1955) are in order. While the A.N.A.R.E. material was being examined, the author also had the opportunity to see a large number of specimens from CSIRO Sta. 76/54 at 39° 30′ S, 144° 30′ E in Bass Strait. These were clearly conspecific with the A.N.A.R.E. material. They showed rather more variation in depth of serration of the uropod rami and in the presence or absence of minute serrulation on the peduncle of uropod 3 than might have been expected; in addition, the males had the characteristic peraeopod 1 illustrated for *P. australis* (Hurley, 1955: fig. 179) whilst the females had the typical *gracilipes* "long-legged" form (Bowman,

1960: fig. 14a). This does not contradict the previous assertion that *P. australis* can be recognised by "coarse, very definite and deep" serration of the third uropod rami. It does, however, indicate the need for caution in identifying these species and for further work on *P. australis.*

Hyperiella dilatata Stebbing, 1888.

Stebbing, 1888: 1403–1407, pl. 171; Spandl, 1927: 162, fig. 5; Barnard, 1930: 413–414; Barnard, 1932: 274–275, fig. 161.

OCCURRENCE: Sta. 60–63, 1 ovigerous female, 4½ mm; Sta. 64–65, 1 juvenile, 2 mm.

REMARKS: The author's attention was drawn to an astonishing similarity, particularly in gnathopods (compare Walker, 1907, pl. 1, and Spandl, 1927, fig. 5) and epimeral plates, between the published figures of this species and Walker's *Hyperia macronyx* from Antarctic waters (Walker, 1907: 7–8, pl. 1). It was tempting to think the two species were the same, although Barnard and Walker each recorded both species without drawing attention to this similarity.

Fortunately, the author was also able to examine a small collection of Antarctic Hyperiids collected by Mr Jack Littlepage at McMurdo Sound. Amongst numerous *Hyperiella dilatata* from a number of stations were occasional *Hyperia macronyx.* The former can be recognised by the spider-like spread of the peraeopods, well shown in Stebbing (1888, pl. 171) and by the generic character which separates *Hyperia* from *Hyperiella* – the third peraeopod is only as long as the fourth in *Hyperia* but noticeably longer in *Hyperiella.* Most characteristic, however, is the strong acute anterodistal spur on segments 2, 3, and 4 of Pr. 3–5, well shown in Stebbing's figures. This angle is rounded in *H. macronyx.*

DISCUSSION

It has been suggested that *Parathemisto gracilipes* is a neritic species (Hurley, 1955) which prefers warmer waters than *P. gaudichaudii* (Bary, 1959; Bowman, 1960). The present data (Fig. 1) suggests that, if neritic, it nevertheless extends well out to sea, but its distribution does not overlap *P. gaudichaudii* except at its southernmost station, Sta. 92 (48° 52′ S, 152° 43′ E). *P. gracilipes* definitely prefers warmer water than *P. gaudichaudii* and the distribution of both species would appear to be controlled by the Subtropical Convergence Region (Burling, 1961). The southernmost occurrence of *P. gracilipes* in this material was barely south of the southernmost limit of the generalised Convergence Region postulated by Burling and also represents the northernmost occurrence of *P. gaudichaudii.*

The distribution of *Hyperiella dilatata* is of interest. Barnard (1932) has pointed out that with two exceptions (Spandl, 1927 – 58° 29′ S, 89° 58′ E, and Barnard, 1932 – 50° 30′ S, 5° 34′ E), *Hyperiella dilatata* has not been reported north of 60° S where it is apparently replaced by *Hyperiella antarctica.* The present data support this conclusion. In looking for controlling factors, one is inclined to look to the Antarctic Convergence but this con-

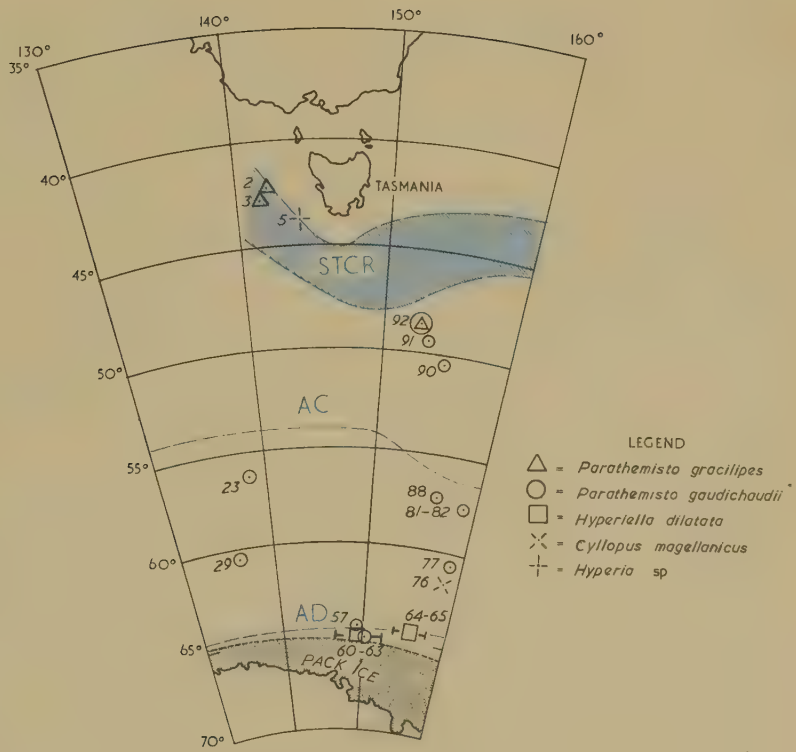

FIG. 1—Distribution of some pelagic Hyperiidea collected by "Magga Dan" between Australia and Antarctica.

STCR = Subtropical Convergence Region (after Burling, 1961).
AC = Antarctic Convergence (estimated annual mean position)–after Mackintosh, 1946).
AD = Antarctic Divergence (after U.S.H.O. Oceanographic Atlas, 1957).
Pack-Ice = Pecked line is estimated mean position of edge of pack-ice for February (after Mackintosh, 1946; U.S.H.O. Oceanographic Atlas, 1957).

trol appears to be too far to the north. The boundary accords rather better with the northern limits of the pack-ice although it does not exactly coincide. However, a further hydrological feature has recently been defined south of the Convergence which fits the situation rather better. This is the Antarctic Divergence which occurs just north of the northern limit of the pack-ice surrounding Antarctica. "Between New Zealand and about 64° S, westerly winds cause a general eastwards motion in water at all depths, and south of 64° S the mean winds are easterlies and there is a drift of water to moderate depths. Since near-surface waters in the 'Ekman' layer move to the left of the wind direction, there is upwelling near 64° S known as the Antarctic Divergence" (Burling and Garner, 1959) (see also U.S. Navy Hydrographic Office "Oceanographic Atlas" (1957); and Wexler (1959)). Thus, the resultant surface current direction is to the north-east north of the Divergence and south-west south of it.

There is evidence from other groups also that the Divergence acts as a control on distribution. Johns (1936) found that *Euphausia frigida* and *Euphausia triacantha* were confined to an area between the Antarctic Convergence and a line just north of the pack-ice whereas *Euphausia superba* came no further north than just outside the edge of the pack-ice. Baker (1959) has confirmed that, in general, the latter two species are "confined to different water masses, *E. triacantha* to the West-Wind Drift and *E. superba* to the East Wind and Weddell Drifts. The mixing is only found at the junction of these water masses and is most marked in the region of South Georgia where . . . hydrological conditions are complex".

On the basis of phytoplankton distribution Hart (1942) divided the Antarctic into three main biogeographical regions, Northern, Intermediate, and Southern. The boundary between the Intermediate and Southern Regions was originally defined in terms of distance from the ice edge but in his 1942 paper this definition is abandoned for an "arbitrary one, placing its northern limit at the Antarctic Circle" (66° 30′ S). Thus, the fact of a faunal barrier fringing the pack-ice has been established for some time; it has remained for the physical definition of the Divergence to supply a hydrological explanation for it.

Other factors as well as the mere physical one of a divergence are, of course, likely to be operative. The distribution of *Hyperiella dilatata,* replaced north of 60° S by *Hyperiella antarctica,* may for instance be considerably influenced by salinity effects from the pack-ice melt-water.

While the data is too limited to assert without fear of contradiction that a control is exerted on northward distribution of this species by the Antarctic Divergence rather than the Convergence, the evidence points in that direction.

Bowman (1960) indicates that *P. gaudichaudii* comes no further north than about 44° S in the Tasmanian region and Hurley (1955) has suggested it is restricted to deep water. The present data are in agreement.

The southern limit of *Parathemisto gaudichaudii* is uncertain. Some previous records (e.g., Barnard, 1932) suggest that it might occur south of the Antarctic Divergence but much closer correlation of the position of the Divergence with that of the specimens in question at the time of their cap-

ture is required to establish the point. On the other hand, Mackintosh (1934), discussing the distribution of the macroplankton in the Atlantic sector of the Antarctic, characterises *Parathemisto gaudichaudii* as a "warm water species sometimes found in colder regions". Since he is dealing particularly with the South Georgia region, this may indicate that *P. gaudichaudii* is limited in its southward distribution by the Divergence. Significantly, when Mackintosh comes to draw up macroplanktonic faunal boundaries he establishes a "transition belt" – "in which the normal limits of the warmest water species and the northern limits of the coldest water species are found". This lies between about 63° and 64° S in the Bellingshousen Sea. It is deflected farther north by the Graham Land Peninsula in the Eastern Scotia Sea although here, at a distance of 1° to 2° farther north, it parallels the line of the pack-ice. Thus, it is possible that the Divergence does delimit the southern boundaries of *P. gaudichaudii* which, as one might expect on theoretical grounds may not cross the Divergence. .

To the north, it is tempting to look for correlations between the various forms of *P. gaudichaudii* and its distribution either side of the Antarctic Convergence. David (1955) has shown the existence of two such races in the chaetognath *Sagitta gazellae,* corresponding to Antarctic and Subantarctic Surface Waters. However, neither A.N.A.R.E. nor Banzare material (Hurley, 1960) gives support to any such correlation of the known morphological varieties of *P. gaudichaudii* which are more probably growth stages (Hurley, 1960). Nevertheless, the point is worth remembering when larger numbers of specimens from each side of the convergence are available for examination.

On the basis of these points it seems likely that *P. gracilipes, P. gaudichaudii,* and *Hyperiella dilatata* are characteristic respectively of warm water (perhaps of neritic or mixed origin) north of or in the Subtropical Convergence Region, oceanic cold water south of the Subtropical Convergence Region, and colder water south of the Antarctic Divergence.

Acknowledgments

The A.N.A.R.E. collection was kindly made available by Mr David Tranter, of the CSIRO Marine Biological Laboratory at Cronulla, Australia. Specimens of *Parathemisto gracilipes* from Sta. DH 76/54 were made available by Dr W. Dall of Cronulla, and Antarctic Hyperiids from the collections of Mr Jack Littlepage of Stanford University, under the United States Antarctic Research Programme at McMurdo Sound, were also examined.

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A NEW ZEALAND PHYTOCHEMICAL SURVEY

PART 2. THE DICOTYLEDONES

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Summary

697 species or varieties of dicotyledones of the New Zealand flora have been examined for the presence of alkaloids, leucoanthocyanins, saponins, and triterpenes or steroids. Tests have been carried out where possible on all available parts of fresh material but recourse to the use of dried herbarium samples has been widely made. The results are intended as a guide for future work.

INTRODUCTION

In Part 1 (Cain, Scannell and Cambie, 1961) the results of testing gymnosperms endemic to New Zealand for the presence of alkaloids, leucoanthocyanins, saponins, and triterpenes or steroids were reported. With the recent publication of a new manual of the New Zealand flora (Allan, 1961) embracing many changes in botanical classification, the results of chemical testing of the New Zealand dicotyledones are now reported.

As in Part 1 the methods of testing for alkaloids, saponins, and triterpenes or steroids follow the surveys carried out on the Australian flora (Webb, 1949, 1952; Simes, Tracey, Webb and Dunstan, 1959) and that of Papua - New Guinea (Webb, 1955) while that for leucoanthocyanins follows the survey by Bate-Smith and Metcalfe (1957). A recent survey for the presence of alkaloids in the Hong Kong flora (Arthur and Cheung, 1960) cites further similar surveys carried out in the United States, Russia, Argentina, North Borneo, Malaya, and Hawaii.

The dicotyledonous plants tested here include fresh plant samples collected mainly from the Auckland or central North Island areas. A limited number of samples collected from the South Island and the Kermadec Islands are included. In addition, in order to test a representative selection of the New Zealand flora, many dried samples from the herbarium of the Auckland Institute and Museum have been used. In all, 697 species or varieties of families representing approximately 45% of the known dicotyledones have been tested, and the results are reported in Table 1. Where possible, especially with fresh samples, all parts of the plant with the exception of the roots have been examined. By preference to lists in alphabeticaal order as used in other similar surveys, families and species are listed in Table 1 as in Allan (1961), in the hope that taxonomic correlations may be more evident to the botanist and chemist. Allan (1961) is accepted as authority for botanical nomenclature.

The methods used in testing have been given in detail in Part 1 of this

series and are not repeated here. In the case of many herbarium samples where the minimum of plant material was used in order not to destroy the samples, tests for alkaloids have been carried out using Dragendorff's reagent only. While the use of a single alkaloid precipitating reagent can lead to spurious and inconclusive results, of all the precipitating reagents used (Cromwell, 1955) Dragendorff's has been found to be the most reliable and in only a few cases was a positive test given by Mayer's reagent which was not also given by Dragendorff's reagent.

KEY TO ABBREVIATIONS IN TABLE 1

The results of the tests are given in the following order and abbreviated form:

SYSTEMATIC NAME OF PLANT

The name used in Allan (1961) is given. Species given by Cheeseman (1925) which do not appear in Allan have been rejected as have also the majority of less well authenticated hybrid species.

MAORI NAME

The Maori name is that given by Anderson (1926) and by Allan (1961). Where the Maori name is unknown or non-existent, the popular name given by Anderson is recorded.

PLANT PART

B = bark, F = fruit, Fl = flowers, H = whole plant or herb, L = leaves, R = root, RB = root bark, Rh = rhizome, RW = root wood, S = seeds, T = twigs, W = wood.

LOCALITY

General area of collection of the fresh plant. "Auckland" refers to samples collected from the grounds of the University of Auckland or the Auckland city and suburban areas. "Herbarium" refers to a sample from the herbarium of the Auckland Institute and Museum. Details of the particular locality of collection of both fresh plant material and of herbarium samples tested are recorded in the card index system used in the compilation of Table 1 and now lodged in the B.E.C.C.S. Laboratory, Cornwall Hospital.

MONTH COLLECTED

Month of actual collection in the field is given. Testing was carried out in most cases within a few days of collection. In some cases, however, considerable time, up to 6 months, elapsed before testing. This has not been found to be markedly detrimental to the quality of the results in a number of test cases, e.g., a sample of *Pomaderris kumerahou* still gave strong saponin froth tests after 15 years of storage.

HERBARIUM NUMBER

For herbarium samples the Auckland Institute and Museum number of a leaf specimen tested is recorded in all cases. In certain cases leaf specimens

of fresh samples actually tested have been lodged in the herbarium to aid future identification, and their number is recorded.

ALKALOID TEST

M = Mayer's reagent, D = Dragendorff's reagent. Precipitates are classified as weak (+), medium (++), and strong (+++) positives or negative (—).

LEUCOANTHOCYANIN TEST

LA = Leucoanthocyanin. Tests are classified as positive (+) or negative (—).

SAPONIN TEST

Sap. = Saponin. Tests are classified as positive or negative.

LIEBERMANN-BURCHARD TEST

LB = Liebermann-Burchard. Tests are classified as positive or negative.

RESULTS AND DISCUSSION

The results of testing are recorded in Table 1. Full discussions of the methods used, the limitations of the tests and of the use of herbarium samples for testing have been made by Webb (1949, 1952, 1955), Simes *et al.* (1959) and by Bate-Smith and Metcalfe (1957). The results of any previously recorded testing on New Zealand species or published work on the isolation of the types of compound under consideration are reported as footnotes throughout Table 1.

Although only Dragendorff's reagent was used in testing for alkaloids in many herbarium samples, and consequently some alkaloid bearing species may have been missed, Table 1 illustrates the lack of alkaloid potentialities of the New Zealand flora.

Alkaloids were found to be mainly present in the following families: Aizoaceae, Apocynaceae, Chloranthaceae, Cruciferae, Monimiaceae, Papilionaceae, Piperaceae, Ranunculaceae, Rutaceae, Solanaceae, and Violaceae. They were also present in the families, Avicenniaceae, Corynocarpaceae, Nyctaginaceae, Myoporaceae, and Passifloraceae in which either only a single New Zealand species exists or a single species was examined. Alkaloids also appeared to be scattered in the families Compositae, Scrophulariaceae and Umbelliferae. Generally the New Zealand flora contains few species in well authenticated alkaloid-bearing families, e.g., Aizoaceae, Apocynaceae, Monimiaceae, Piperaceae, Rutaceae, and Solanaceae. Consequently little chemical work on alkaloids of the New Zealand flora has been carried out. Moreover, although there are many New Zealand species of the Australian and European alkaloid-bearing families, Compositae, Papilionaceae, Ranunculaceae, and Violaceae, the alkaloid content appears to be variable and when present it usually occurs in only small amount.

The test for leucoanthocyanins is characteristic and can rarely be misinterpreted. Bate-Smith and Metcalfe have shown that leucocyanidin and leucodelphinidin are the two leucoanthocyanins which usually occur naturally but

no attempt has been made in the present work to differentiate between these two by chromatography of the derived anthocyanidins. Of the families well represented in the New Zealand flora the following were usually found to contain leucoanthocyanins: Cornaceae, Cunoniaceae, Droseraceae, Elaeocarpaceae, Epacridaceae, Ericaceae, Escalloniaceae, Fagaceae, Lauraceae, Loranthaceae, Meliaceae, Myrsinaceae, Myrtaceae, Polygonaceae, Proteaceae, Rhamnaceae, Rosaceae, Sapindaceae, Thymelaeaceae, and Winteraceae. A close agreement is evident from a comparison of these families with those recorded as tanniniferous by Bate-Smith and Metcalfe. These authors have discussed the relationship between occurrence of leucoanthocyanins and tannins and concluded from their survey that leucoanthocyanins show a tendency to be more frequently present in woody plants.

Little consistency among species of any one family was observed for positive saponin tests. Saponins appeared, however, to be most abundant in the families: Araliaceae, Myrsinaceae, Onagraceae, Rhamnaceae, Schrophulariaceae, and Solanaceae. Only species which give froths stable for at least 30 min are recorded in Table 1. A number of other species gave weak froth tests and may contain small amounts of saponins. In the case of herbarium samples, some of which were forty or more years old, a negative saponin test should be interpreted with reservations. Decomposition of saponins on drying and storage of plant material might well be expected to occur, and the testing of fresh material could show a greater abundance of saponin-containing species.

The Liebermann-Burchard test for triterpenes or sterols is considered to be the least reliable of all the tests. The test is limited by a number of factors chief of which is the masking of colorations by the green of chlorophyll, especially in leaf tissue, and by the dark colours of many plant extracts. A high concentration of triterpene or steroid in crude extracts was normally found necessary in order to observe a positive test. In addition many cases were observed where the crude extract from a plant failed to give a satisfactory positive test, but a subsequently purified fraction then gave a positive test. Moreover a positive test may be due to the presence of a polycyclic substance other than triterpene or steroid while most saturated steroids or triterpenes would not be detected by this method. Families which appeared to be rich in triterpene or steroidal material are: Araliaceae, Compositae, Epacridaceae, Ericaceae, Myrsinaceae, Myrtaceae, Onagraceae, Pittosporaceae, and Solanaceae.

Finally it is pointed out that the results of testing reported here should be considered as a guide only to the presence of alkaloids, leucoanthocyanins, saponins, and triterpenes or steroids in the New Zealand flora. Confirmation of both positive and negative tests can come only from complete and systematic chemical investigation of each species by extraction and either isolation of constituents or demonstration of their presence by chromatographic methods. It is the hope of the present authors that Table 1* will provide a basis for further work and give an indication of species which may afford fruitful sources of each type of compound for further studies.

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*A similar study of the New Zealand monocotyledones is in progress.

TABLE 1

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
WINTERACEAE										
Pseudowintera axillaris Dandy	horopito	L	Herbarium	—	24475	—	—	—	+	—
		W	,,	—	,,	—	—	+	+	—
Pseudowintera colorata Dandy	oramarama	L	Ohakune	Oct.	50087	—	—	—	—	—
		W	,,	,,		—	—	+	—	—
		B	,,	,,		—	—	+	—	—
Pseudowintera traversii Dandy		L, T	Herbarium	—	4379	—	—	+	—	—
LAURACEAE										
Beilschmiedia tarairi Benth. et Hook. f.	taraire	L	Karekare	March	46472	—	—	—	—	—
		W	,,	,,		—	—	+	+	—
		B	,,	,,		—	—	+	+	—
		RB	,,	,,		—	—	+	+	—
		F	Auckland	April		—	—	—	—	—
Beilschmiedia tawa Benth. et Hook. f.	tawa	L	Waitakeres	March		—	—	+	—	—
		W	,,	,,		—	—	+	+	—
		RW	,,	,,		—	—	+	+	—
		B	,,	,,		—	—	+	—	—
		RB	,,	,,		—	—	+	—	—
Litsea calicaris Benth. et Hook. f.	mangeao	L	Auckland	July		—	—	—	—	—
		W	,,	,,		—	—	+	—	—
		B	,,	,,		—	—	+	—	—
		RW, RB	,,	,,		—	—	+	—	—
		T	,,	,,		—	—	+	—	—
Cassytha paniculata R. Br.	mawhai	L	Herbarium	—	69206	—	—	+	+	—
		St	,,	—	,,	—	—	+	—	—

MONIMIACEAE										
Hedycarya arborea J.R. et G. Forst.	porokaiwhiria (pigeonwood)	L	Waikato Heads	Jan.		+	+(1)	−	−	−
		W	,,	,,		++	++	+	−	
		B	,,	,,		−	−	+	−	−
		RB	,,	,,		−	−	+	−	
		F	,,	,,		++	++	−	−	−
Laurelia novae-zelandiae A. Cunn.	pukatea	L	Huia	March	46488	++	++	(2)	+	−
		W	,,	,,		+	+	−	+	
		RW	,,	,,		+	+	−	+	
		B	,,	,,		+	+(3)		+	−
		RB	,,	,,		++	++	−	+	−
RANUNCULACEAE										
Ranunculus lyallii Hook. f.	mountain-lily	L	Herbarium	—	14818	−	−	−	−	−
		St	,,	—	,,	+	+			
Ranunculus insignis Hook. f.	korikori	L, St	Herbarium	—	70606	+	+		−	−
		Fl	,,	—	,,	+	+	−		−
Ranunculus monroi Hook. f.		L, St	Herbarium	—	12436		−	−	−	−
		Fl	,,	—	,,	+	+	−		−
Ranunculus godleyanus Hook. f.	yellow mountain-lily	L, St	Herbarium	—	70605	−	−	−	−	−
		R	,,	—	,,	−	−	−	−	−
		Fl	,,	—	,,	−	−	−		−
Ranunculus nivicolus Hook.	Egmont buttercup	L	Mt. Egmont	Dec.			−	−	−	−
		Fl	,,	,,		−	−	−	−	−
Ranunculus clivalis Allan		L, St	Herbarium	—	70607	+	+	−	−	−
Ranunculus novae-zelandiae Petrie		L, St	Herbarium	—	70608	−	−	−	−	−
Ranunculus sinclairii Hook. f.		H	Herbarium	—	14829	−	+	−	−	
Ranunculus sericophyllus Hook. f.	silky alpine buttercup	L, St	Herbarium	—	14827	+	+	−	−	−
		Fl	,,	—	,,	−	+	−	−	−
Ranunculus hirtus Banks et Sol.	maruru	H	Herbarium	—	22791	+	+	−		
Ranunculus urvilleanus Cheesem.		L, St	Herbarium	—	12414	−	−	−	−	−

(1)E. P. White (pers. comm.) has shown the presence of alkaloids (0.02%) in the leaves.
(2)Bate-Smith and Metcalfe (1957) record the presence of leucoanthocyanins in the leaves.
(3)Aston (1910) has reported the isolation of the alkaloids, pukateine, laureline, and laurepukine from the bark. Their constitution and pharmacological activity have been reported by other workers: Girardet (1931a, 1931b), Barger and Girardet (1931, 1932), Schlittler (1932), Fogg (1935).

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
Ranunculus acaulis Banks et Sol.	shore buttercup	H	Herbarium	—	70609	+	+	—	—	—
Ranunculus porrectus Simpson		H	Herbarium	—	22889	—	—	—	—	—
Ranunculus macropus Hook. f.	raoriki	H	Herbarium	—	1441	—	—	—	—	—
Ranunculus rivularis Banks et Sol.	water buttercup	H	Herbarium	—	35679	—	—	—	—	—
Caltha novae-zelandiae Hook. f.	Maori marigold	H	Herbarium	—	14838	+	+	—	—	—
Clematis paniculata Gmel.	puawhananga	L	Waitakeres	Aug.		+	+	—	—	—
		St, Fl	„	„		++	++	—	—	—
Clematis foetida Raoul		L, St	Herbarium	—	2221	+	+	—	(+)	—
		Fl	„	—	„	+	+	—	—	—
Clematis parviflora A. Cunn.	ngakaukiore	L, St	Herbarium	—	50383	—	—	—	—	—
		Fl	„	—	„	+	+	—	—	—
Clematis asutralis Kirk		L	Herbarium	—	44056		—	—	—	—
		St	„	—		—	+	—	—	—
		Fl	„	—		—	—	—	—	—
Clematis australis kirk var. *rutifolia* Allan		L, St	Herbarium	—	46054	—	+	—	—	—
		Fl	„	—	„	+	+	—	—	—
Clematis hookeriana Allan	puataua	L, T	Taupo	Oct.	46542		—	—	—	—
		W, B	„	„			—	—	—	—
Clematis afoliata Buchan.	leafless clematis	St, Fl	Herbarium	—	14814	+	+	—	—	—
Clematis marata Armst. f.		L, St	Herbarium	—	1513		—	—	—	—
CHLORANTHACEAE										
Ascarina lucida Hook. f.	hutu	L	Auckland	Sept.		—	+	—	—	—
		W	„	„		—	+	—	—	—
		Fl	„	„		—	+	—	—	—

Ascarina lucida Hook. f. var. *lanceolata* Allan		W	Kermadec Is.	June		−	+	−	−	−
		B	„	„		−	+	−	−	−
PIPERACEAE										
Macropiper excelsum Miq.	kawakawa	L	Waikato Heads	Jan.		++	++	−(4)	−	−
		W	„	„		+	+	−	−	−
		B	„	„		+	+	−	−	−
		RW, RB	„	„		+	+	−	−	−
		F	„	„		+	+	−	−	−
Macropiper excelsum Miq. var. *majus* Allan		L	Tiritiri	Sept.		++	++	−	−	−
Peperomia tetraphylla Hook. et Arn.		L, St	Herbarium	—	28061	+	+	−	−	−
Peperomia urvilleana A. Rich.		L	Waitakeres	March		++	++	−	−	−
CRUCIFERAE										
Lepidium oleraceum Forst. f.	nau	L	Herbarium	—	70601	+	+	−	−	−
		St	„	—	„	+	+	−	−	−
Lepidium flexicaule Kirk		L, St, S	Herbarium	—	14865	+	+	−	−	−
Lepidium sisymbrioides Hook. f.	pepperwort	L	Herbarium	—	15769	+	+	−	−	−
		W, B	„	—	„	+	+	−	−	−
Lepidium matau Petrie		H	Herbarium	—	70600	+	+	−	−	−
Notothlaspi rosulatum Hook. f.	penwiper plant	H	Herbarium	—	14867	+	+	−	−	−
Notothlaspi australe Hook. f.		H	Herbarium	—	14869	−	+	−	+	−
Cardamine debilis Banks ex DC.	panapana	H	Herbarium	—	70602	−	+	−	−	−
Cheesemania wallii Allan		L, St	Herbarium	—	1513	+	+	−	+	−
Cheesemania enysii Schulz		L, St, Fl	Herbarium	—	14864	+	+	−	+	−
Rorippa stylosa Allan	matangoa	L, St	Herbarium	—	70603	+	+	−	−	−
VIOLACEAE										
Viola filicaulis Hook. f.	slender violet	H	Herbarium	—	70604	+	+	−	+	−
Viola cunninghamii Hook. f.	Maori violet	H	Herbarium	—	796	−	+	−	−	−
Viola lyallii Hook. f.	haka	H	Herbarium	—	798	+	+	−	−	−

(4)Bate-Smith and Metcalfe (1957) record the absence of leucoanthocyanins in the leaves.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
Melicytus macrophyllus A. Cunn.	large-leaved whitewood	L	Auckland	Sept.		−	+	−	−	−
		W	,,	,,		−	+	−	−	−
Melicytus ramiflorus J. R. et G. Forst.	mahoe	L	Waitakeres	Jan.	50077	+	+	−(5)	−	−
		W	,,	,,		+	++	−	−	−
		RW	,,	,,		+	+		−	−
		B	,,	,,		+	+	−	−	−
		RB	,,	,,		+	+	−	−	−
		L	Ohakune	Oct.	50086	+	+	−	−	
		W	,,	,,		+	++	−	−	−
		Fl	Waitakeres	Aug.		+	++	−	−	−
Melicytus lanceolatus Hook. f.	mahoewao	L	Auckland	May		++	++	−	+	+
		W	,,	,,		+	++	−	+	−
		B	,,	,,		+	+	−	+	−
Melicytus micranthus Hook. f.	manakura	L	Auckland	Aug.		+	+	−	−	−
		W, B	,,	,,		+	+	−	−	−
		L	Herbarium	—	51575	+	+	−	−	−
		T	,,	—	,,	+	+	−	−	
Hymenanthera novae-zelandiae Hemsl.		L	Auckland	Sept.		+++	+++	−	(+)	−
		W	,,	,,		+	++	−	−	
Hymenanthera chathamica Kirk	Chatham mahoe	L	Herbarium	—	29702	+	+	−(6)	+	
		W	,,	—	,,	+	+	−	+	−
		Fl	,,	—	,,	++	++	−	−	−
Hymenanthera angustifolia R. Br.		L	Waimarino	Jan.		+	+	−		−
		W	,,	,,		+	+			−
Hymenanthera crassifolia Hook. f.	thick-leaved hymenanthera	L	Herbarium	—	15035	++	++			−
		W	,,	—	,,	+	+	−		
Hymenanthera obovata Kirk		L	Herbarium	—	355	+	+	−(7)	+	−
		W	,,	—	,,	+	++	−	+	
		Fl	,,	—	,,	−	+	−	−	+

DROSERACEAE										
Drosera arcturi Hook.	alpine sundew	H	National Park	Jan.			–	+	–	
Drosera stenopetala Hook. f.	sub-antarctic sundew	H	Herbarium	—	44099		–	+		
Drosera pygmaea DC.	pigmy sundew	H	Herbarium	—	14883		–	+	–	–
Drosera spathulata Labill.	spoon-leaved sundew	H	National Park	Jan.		–	–	+	–	
Drosera binata Labill.	scented sundew	H	Herbarium	—	44415	–	–	+(8)	–	–
Drosera auriculata Backh.	climbing sundew	H	Herbarium	—	271	–	–	+	–	
AIZOACEAE										
Disphyma australe J. M. Black	horokaka	H	Piha	May			–	+	–	
Tetragonia tetragonioides Kuntze	kokuhi	H	Piha	May	46512	++	++(9)	–	+(10)	–
	(rengamutu)	L	Herbarium	—	2349	+	+	–	+	
Tetragonia trigyna Banks et Sol.	beach-spinach	L	Herbarium	—	14789	+	+	–	+	–
		St	„	—	„	–	–	–	+	–
CARYOPHYLLACEAE										
Spergularia marginata Kittel	salt-marsh sand-spurrey	H	Herbarium	—	14804	–	–	–	–	
Stellaria roughii Hook. f.	Rough's chickweed	H	Herbarium	—	14799	–	+	–	–	–
Stellaria gracilenta Hook. f.	Maori chick-weed	H	Herbarium	—	14802	–	+	–	–	–
Stellaria parviflora Banks et Sol.	stitchwort	L, St	Herbarium	—	14797	–	+	–	–	–

(5)Bate-Smith and Metcalfe (1957) record the absence of the leucoanthocyanins in the leaves.
(6)Bate-Smith and Metcalfe (1957) record the absence of leucoanthocyanins in the leaves.
(7)Bate-Smith and Metcalfe (1957) record the absence of leucoanthocyanins in the leaves.
(8)Bate-Smith and Metcalfe (1957) record the presence of leucocyanidin in the leaves.
(9)Webb (1949, 1952) records positive alkaloid tests, and Hurst (1942) reports positive alkaloid tests by Finnemore *et al.* for *T. expansa* Murr. (= *T. tetragonioides*.)
(10)Murray (1950) reports the probable presence of appreciable amounts of saponins. Greshoff (1909) found the shoots contain much saponin.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
Colobanthus acicularis Hook. f.		L, St	Herbarium	—	70605	—	—	—	—	—
Colobanthus affinis Hook. f.		H	Herbarium	—	14792	—	+	—	+	—
Hectorella caespitosa Hook. f.	tufted hectorella	H	Herbarium	—	61886	—	—	—	—	—
Scleranthus biflorus Hook. f.	naereere	H	Herbarium	—	14807	—	—	—	+	—
ELATINACEAE										
Elatine gratioloides A. Cunn.	water-wort	H	Herbarium	—	319	—	—	—	—	—
PORTULACACEAE										
Claytonia australasica Hook. f.		H	National Park	Jan.		—	—	—	—	—
Montia fontana L.		H	Herbarium	—	163	—	+	—	—	—
POLYGONACEAE										
Muehlenbeckia astonii Petrie	shrubby pohuehue	L	Auckland	Sept.		—	—	+	—	—
		W, B	„	„		—	—	+	—	—
Muehlenbeckia ephedroides Hook. f.		St	Herbarium	—	70606	—	—	+	+	—
Muehlenbeckia axillaris Walp.	creeping pohuehue	W, B	Herbarium	—	50067	—	—	+	—	—
Muehlenbeckia australis Meissn.	broad leaved pohuehue	L	Taupo	Oct.	46539	—	—	+	—	—
		W	„	„		—	—	+	—	—
		B	„	„		—	—	+	—	—
Muehlenbeckia complexa Meissn.	pohuehue	L, St	Huia	March	46483	—	—	+	+	—
Polygonum decipiens R. Br.	tutunawai	H	Herbarium	—	1010	—	—	+	+	—
Rumex flexuosus Sol. ex Hook. f.	runa	L	Herbarium	—	14772	—	—	+	—	—
		St	„	—	„	—	—	+	+	—
AMARANTHACEAE										
Alternanthera denticulata R. Br.		L, St, Fl	Herbarium	—	35864	—	—(11)	—	+(12)	—

CHENOPODIACEAE										
Chenopodium allanii Aellen	poipapa	L, St	Herbarium	—	61603		—	—	+(13)	—
Chenopodium ambiguum R. Br.	oak-leaved goose-foot	L, St	Herbarium	—	14780		—	—	+(13)	—
Atriplex buchananii Cheesem.		L, St	Herbarium	—	14782		—	—	—	—
Theleophyton billardieri Moq.	New Zealand orache	L, St	Herbarium	—	44890		—	—	—	—
Suaeda novae-zelandiae Allan	sea-blite	H	Auckland	April		—	—	—	—	—
Rhagodia triandra Aellen		L, St	Herbarium	—	15777		—	—	+	—
Salicornia australis Sol. ex Benth.	austral glasswort	H	Mangere	Jan.		—	—	—	—	—
GERANIACEAE										
Geranium sessiliflorum Cav.	short-flowered cranesbill	H	Herbarium	—	50518	—	—	—	—	—
Geranium microphyllum Hook. f.	small-leaved cranesbill	H	Mt. Egmont	Dec.		—	—	—	—	—
Geranium traversii Hook. f.	Chatham cranesbill	L, St	Herbarium	—	70607	—	—	—	—	—
Pelargonium inodorum Wild.	kopata	L, St, Fl	Herbarium	—	30497	—	—	—	+	—
OXALIDACEAE										
Oxalis lactea Hook.	tutaekaahu (white oxalis)	H	Herbarium	—	24046	—	—	+	—	—
Oxalis stricta L.		H	Piha	May		—	—	—	—	—
Oxalis corniculata L.	yellow oxalis	H	Herbarium	—	26660	—	+(14)	—	—	—
LINACEAE										
Linum monogynum Forst. f.	rauhuia	L, St, Fl	Herbarium	—	14976		—	—	—	—

(11)Webb (1952) records positive alkaloid tests.
(12)Simes *et al.* (1959) record negative saponin tests.
(13)Murray (1950) reports the probable presence of appreciable amounts of saponins.
(14)Webb (1949) records negative alkaloid tests.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
HALORAGACEAE										
Haloragis erecta Eichl.	toatoa	L	Auckland	Nov.		—	—	—	(+)	—
		St	„	„		—	—	+	(+)	—
Haloragis cartilaginea Cheesem.		L	Herbarium	—	50384	—	—	+	—	—
		St	„	—	„	—	—	+	—	—
Haloragis depressa Walp.		H	Herbarium	—	50833	—	—	+	—	—
Haloragis incana Walp.	piripiri	L, St	Herbarium	—	70608	—	—	—	—	—
Haloragis procumbens Cheesem.	piripiri	L, St	Herbarium	—	35720	—	—	+	+	—
Gunnera monoica Raoul	solitary gunnera	L, St	Herbarium	—	65417		—	—	—	
Gunnera strigosa Col.		H	Herbarium	—	336		—	—	(+)	—
Gunnera prorepens Hook. f.	creeping gunnera	H	Herbarium	—	61896		—	—	—	—
Gunnera densiflora Hook. f.		H	Herbarium	—	22398		—	—	—	—
Gunnera dentata Kirk	red-fruited gunnera	H	Herbarium	—	58906		—	—	—	—
Gunnera arenaria Cheesem.	sand gunnera	H	Herbarium	—	298		—	—	(+)	
Myriophyllum votschii Schindler	small water-milfoil	H	Herbarium	—	59048		—	+	—	—
Myriophyllum pedunculatum Hook. f.		H	Herbarium	—	70609		—	+	—	—
Myriophyllum robustum Hook. f.	stout water-milfoil	L, St	Herbarium	—	1026		—	—	—	—
Myriophyllum propinquum A. Cunn.		H	Herbarium	—	718		—(15)	—	—	—
Myriophyllum elatinoides Gaud.	water-milfoil	H	Herbarium	—	70610		—	—	—	
ONAGRACEAE										
Epilobium nummularifolium R. Cunn.	creeping willow-herb	L, St	Herbarium	—	1959		—	—	+	+

Epilobium pedunculare A. Cunn.	long stemmed willow-herb	L, St	Herbarium	—	70613		—	—	+	+
Epilobium nerterioides A. Cunn. var. *minimum* Ckn.	short-stemmed willow-herb	Fl	Herbarium	—	70614		—		+	
Epilobium macropus Hook.	mountain water willow-herb	L, St	Herbarium	-	48832		—			+
Epilobium linnaeoides Hook. f.	forest willow-herb	L, St	Herbarium	—	26553		-	-		
Epilobium rotundifolium Forst. f.	round leaved willow-herb	H	Waitakeres	March	46460	—	—			-
Epilobium tenuipes Hook. f.		H	Herbarium	—	1444		—	—	+	+
Epilobium tasmanicum Hauss.		L, St	Herbarium	-	448		—	-		+
Epilobium cockaynianum Petrie		L, St	Herbarium	—	70614a		-	-	+	—
Epilobium chloraefolium Hauss.	mountain willow-herb	L, St	Herbarium	-	1929		-	—	-	+
Epilobium wilsonii Petrie var. *pallidum* Simpson et Thomson		L, St	Herbarium	—	70615		-	—	+	+
Epilobium crassum Hook. f.	thick leaved willow-herb	L	Herbarium	—	50287		—	—	-	+
		St	„	—	„		-	-	+	+
Epilobium brevipes Hook. f.		L, St	Herbarium	—	69470		-	—	+	—
Epilobium melanocaulon Hook.		L, St	Herbarium	—	24232		-	-	(+)	
Epilobium microphyllum A. Rich.	papakoura	H	Herbarium	—	66726		—	—	—	—
Epilobium glabellum Forst. f.	glossy leaved willow-herb	L, St	Herbarium	—	69469		—	—	+	
Epilobium pallidiflorum Sol. ex A. Cunn.	swamp willow-herb	L, St	Herbarium	—	35480	—	—	-	+	
		Fl	„	—	„		-	—	+	+
Epilobium erectum Petrie	tall willow-herb	L, St	Herbarium	—	58270		—	—	+	—
Epilobium cinereum A. Rich.	narrow leaved willow-herb	L, St	Herbarium	—	70602		—	-	+	+
Epilobium hirtigerum A. Cunn.		L, St	Herbarium	—	1906			—	+	
Epilobium billardierianum Sér.	red stemmed willow-herb	L, St	Herbarium	-	1920		—	-	+	+
Epilobium chionanthum Hauss.	pale leaved willow-herb	L, St	Herbarium	—	445		—	—	—	-

([15])Webb (1949) records the absence of alkaloids.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
Epilobium pubens A. Rich.	soft leaved willow-herb	L, St	Herbarium	—	24311		–	–	–	–
Fuchsia procumbens R. Cunn. ex A. Cunn.	shore-fuchsia	L, St	Herbarium	—	58384		–	–	–	–
		Fl	,,	—	,,		–	–	–	–
Fuchsia excorticata Linn. f.	kotukutuku	L	Waitakeres	April			–	–	–	–
		W	,,	,,			–	–	–	–
		B	,,	,,			–	–	–	–
Fuchsia colensoi Hook. f.	shrub-fuchsia	L	Auckland	Aug.		–		–	–	–
		W	,,	,,		–		–	–	–
CALLITRICHACEAE										
Callitriche muelleri Sond.	southern water star-wort	H	Herbarium	—	69463				–	–
NYCTAGINACEAE										
Heimerliodendron brunonianum Skottb.	parapara	L	Auckland	Sept.		++	++(16)	–	+	–
		W	,,	,,		+	+	–	+	–
		B	,,	,,		+	+	–	+	–
		S	,,	,,		+	++	–	+	+
THYMELAEACEAE										
Drapetes lyallii Hook. f.	Lyall's drapetes	H	Herbarium	—	44173		–	+	–	–
Drapetes multiflorus Allan		L, St	Herbarium	—	44036		–	+		
Drapetes dieffenbachii Hook.	common drapetes	L, St	Herbarium	—	50542		–	+	–	–
Pimelea longifolia Sol. ex Wiks.	taranga	H	Waitakeres	Oct.		–	–	+	–	–
		W	,,	,,		–	–	+	–	–
		Fl	,,	,,		–	–	+	–	–

Pimelea gnidia Willd.		L	Herbarium	—	69460		—	+	—	—
		W	,,	—	,,		—	+	—	—
		Fl	,,	—	,,		—	+	—	—
Pimelea traversii Hook. f.		L	Herbarium	—	69465		—	+	—	—
		W, B	,,	—	,,		—	+	—	—
Pimelea buxifolia Hook. f.	box-leaved pimelea	L	National Park	Nov.	50126	—	—	+	—	—
		W	,,	,,		—	—	+	—	—
		B	,,	,,		—	—	+	—	—
Pimelea tomentosa Druce	twiggy pimelea	L, T	Auckland	Oct.		—	—	+	—	—
		W	,,	,,		—	—	+	—	—
Pimelea arenaria A. Cunn.	aute-tauranga	L	Herbarium	—	50351		—	+	—	—
		W, B	,,	—	,,		—	+	—	—
Pimelea aridula Ckn.		L	Herbarium	—	69466		—	+	—	—
		W, B	,,	—	,,		—	+	—	—
Pimelea suteri Kirk		L, Fl	Herbarium	—	69462		—	+	—	—
		W	,,	—	,,		—	+	—	—
Pimelea prostrata Willd.	pinatoro	H	Tangawhai	Oct.	56529	—	—	+	—	—
Pimelea prostrata Willd. var. *urvilleana* Meissn.		L	Herbarium	—	35845	—	—	+	—	—
		W	,,	—	,,	—	—	+	—	—
Pimelea lyallii Hook. f.	Lyall's pimelea	L, T	Herbarium	—	44411		—	+	—	—
Pimelea sericeo-villosa Hook. f.		L, St	Herbarium	—	56891		—	+	—	—
PROTEACEAE										
Persoonia toru A. Cunn.	toru	L	Waitakeres	March	46470 &	—	—	+	—	—
		W	,,	,,	46471	—	—	+	—	—
		RW	,,	,,		—	—	+	—	—
		B	,,	,,		—	—	+	—	—
		RB	,,	,,		—	—	+	—	—
		L	Herbarium	—	28032		—	+	—	—
		W	,,	—	,,		—	+	—	—
		S	,,	—	,,		—	+	—	—

([16])Webb (1952) records negative alkaloid tests.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
Knightia excelsa R. Br.	rewarewa	L	Waitakeres	Nov.		–	–	–	–	–
		W	,,	Feb.		–	–	–	+(17)	–
		RW	,,	March		–	–	–	+	–
		B	,,	,,		–	–	+	++	–
		RB	,,	,,		–	–	+	+	–
		F	,,	Nov.		–	–	+	–	–
CORIARIACEAE										
Coriaria arborea Lindsay	tree tutu	L	Kawakawa	Dec.		–	–	–	–	–
		W	,,	,,		–	–	–	–	–
		RW	,,	,,		–	–	–	–	–
		B	,,	,,		–	–	–	–	–
		RB	,,	,,		–	–	–	–	–
		Fl	Waitakeres	Aug.		–	–	–	–	–
Coriaria sarmentosa Forst. f.	tutu	L, T	Herbarium	—	44400		–	–	–	–
Coriaria kingiana Col.		L, St	Herbarium	—	22862		–	–	–	–
Coriaria pteridoides W. R. B. Oliver		L	Herbarium	—	50643		–	–	–	
		W	,,	—	,,		–	–	–	–
		Fl	,,	—	,,		–	–	–	–
Coriaria angustissima Hook. f.	ground tutu	L	Herbarium	—	24331		–	–	–	–
		St	,,	—	,,		–	–	–	
Coriaria plumosa W. R. B. Oliver		L, St	Herbarium	—	50276		–	–	–	–
		S	,,	—	,,		–	–	–	–
Coriaria pottsiana W. R. B. Oliver		L, St, S	Herbarium	—	61891		–	–	–	
PITTOSPORACEAE										
Pittosporum turneri Petrie		L	Herbarium	—	32481	–	–	–	–	–
		W	,,	—	,,	–	–	–	+	–
		S	,,	—	,,	–	–	+	–	–
		H	Auckland	Aug.		–	–	–	–	–

Pittosporum virgatum Kirk		L	Herbarium	—	1966		—	—	—	—
		St	,,	—	,,		—	-	—	—
		Fl	,,	—	,,		—	+	—	—
Pittosporum pimeleoides R. Cunn.		L	Auckland	Sept.		—	—	—	—	—
		St	,,	,,		—	—	—	—	—
Pittosporum anomalum Laing et Gourlay		L	Auckland	Sept.		—	—	—	—	—
		L	,,	,,		—	—	—	-	—
Pittosporum obcordatum Raoul var. *wairoaaensis* Matthews	cohou-cohou	H	Auckland	Aug.		—	—	—	-	—
Pittosporum obcordatum Raoul var. *kaitaiaensis* Laing et Gourlay	cohou-cohou	L	Auckland	Aug.		—	—	—	—	-
		W	,,	,,		—	—	—	—	—
Pittosporum rigidum Hook. f.	shrubby pittosporum	L	Herbarium	—	44275		—	—	—	—
		W	,,	—	,,		—	—	—	—
Pittosporum crassicaule Laing et Gourlay		L	Herbarium	—	24318		—	—	—	—
		W	,,	—	,,		—	—	—	—
Pittosporum divaricatum Ckn.		L	Herbarium	—	70617		—	—	—	—
		W	,,	—	,,		—	—	—	—
Pittosporum tenuifolium Sol. ex Gaertn.	kohuhu	L	Huia	March		—	—	—([18])	—	+
		W	,,	,,		—	—	—	—	—
		B	,,	,,		—	—	—	—	+
		RB	,,	,,		—	—	—	—	+
		F	Auckland	April		—	—	—	—	+
Pittosporum colensoi Hook. f.	rautawhiri	L	Taupo	Oct.	46540	—	—	—	—	—
		W	,,	,,		—	—	—	—	—
		B	,,	,,		—	—	—	—	+
		F	,,	,,		—	—	—	—	—
Pittosporum huttonianum Kirk		L	Auckland	Aug.		—	—	—	+([19])	—
		W	,,	,,		—	—	—	—	—
Pittosporum crassifolium Banks et Sol. ex A. Cunn.	karo	L	Piha	Aug.		—	—	—	(+)([20])	+
		W	,,	,,		—	—	—	—	—
		B	,,	,,		—	—	—	—	+
		F	,,	,,		—	—	—	—	+

([17])Murray (1950) reports the probable presence of appreciable amounts of saponins.
([18])Bate-Smith and Metcalfe (1957) record the absence of leucoanthocyanins in the leaves.
([19])Klein (1932) reports the presence of a saponin, "pittosporin", in the leaves.
([20])Murray (1950) reports the probable presence of appreciable amounts of saponins. Klein (1932) reports the presence of a saponin, "pittosporin", in the leaves.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
Pittosporum ralphii Kirk	Ralph's pittosporum	L	Herbarium	—	58263 &		—	—	—	—
		W	,,	—	58264		—	—	—	+
		Fl	,,	—	,,		—	—	—	—
Pittosporum fairchildii Cheesem.	Three kings karo	L	Auckland	Aug.		—	+	—	—	—
		W	,,	,,		—	(+)	—	—	+
Pittosporum kirkii Hook. f.	Kirk's pittosporum	L	Herbarium	—	44781		—	—	—	—
		W	,,	—	,,		—	—	—	—
		S	,,	—	,,		—	—	+	—
Pittosporum cornifolium A. Cunn.	tawhirikaro	L	Waitakeres	March		—	—	—	+(21)	—
		T	Auckland	Aug.		—	—	—	—	—
Pittosporum ellipticum Kirk		L	Auckland	Aug.		—	—	—	—	—
		W	,,	,,		—	—	—	—	+
Pittosporum ellipticum Kirk var. *decorum* Cheesem.		L	Auckland	Aug.		—	+	—	—	—
		W	,,	,,		—	+	—	—	—
Pittosporum umbellatum Banks et Sol. ex Gaertn.	haekaro	L	Herbarium	—	46526	—	+	—	—	—
		W	,,	—	,,	—	—	—	—	+
		F	,,	—	,,	—	—	—	—	—
Pittosporum eugenioides A. Cunn.	tarata	L	Huia	March		—	—	—(22)	(+)(23)	—
		B	,,	,,		—	—	—	—	—
		RB	,,	,,		—	—	—	—	+
		F	,,	,,		—	—	—	—	—
		L	Lake Rotoaira	Nov.	50101	—	—	—	—	—
		W	,,	,,		—	—	—	—	—
PASSIFLORACEAE										
Tetrapathaea tetrandra Cheesem.	kohia	L	Huia	March		—	—	—	—	—
		W	,,	,,		(+)	+	—	—	—
		B	,,	,,		—	—	—	—	—
		RW, St	,,	,,		—	—	—	—	—
		RB, St	,,	,,		++	++	—	—	—
		R	,,	,,		+	+	—	—	—
		F	,,	,,		++	++	—	—	—

CUCURBITACEAE										
Sicyos angulata L.	mawhai	L, St	Herbarium	—	43869	—	(+)	—	+	—
MYRTACEAE										
Leptospermum scoparium J. R. et G. Forst.	manuka	L	Waikato Heads	Jan.		—	—	+	—	+(24)
		W	,,	Dec.			—	+	—	+
		B	,,	Jan.		—	—	+	—	+
		RW, RB	,,	Dec.		—	—	+	—	+
		Fl	Auckland	Aug.		—	—	+	—	+
Leptospermum ericoides A. Rich.	kanuka	L	Manurewa	May		-	—	+	—	+(25)
		W	,,	,,		-	—	+	—	+
		B	,,	,,		—	—	+	—	+
Metrosideros kermadecensis W.R.B. Oliver	Kermadec pohutakawa	L	Kermadec Is.	June		—	—	+	—	—
		W	,,	,,		-	—	+	—	—
		B	,,	,,		-	—	+	—	—
Metrosideros excelsa Sol. ex Gaertn.	pohutukawa	L	Mayor Is.	Nov.		-	—	+	—	+
		St	,,	,,		-	—	+	—	+
		F	,,	,,		-	—	+	—	+
		L	Kawakawa	Nov.		—	—	+	+	+
		W	Auckland	Jan.		—	—	+	—	+
		RW	Kawakawa	Nov.		—	—	+	—	+
		B	,,	,,		—	—	+	—	+
		RB	,,	,,		—	—	+	+	+
		Fl	,,	Jan.		—	—	+	—	+(26)
Metrosideros robusta A. Cunn.	rata	L	Auckland	Aug.		—	—	+	—	—
		W	,,	,,		—	—	+	—	—
		B	,,	,,		—	—	+	—	+(27)

(21)Klein (1932) reports the presence of a saponin "pittosporin", in the leaves.
(22)Bate-Smith and Metcalfe (1957) record the absence of leucoanthocyanins in the leaves.
(23)Murray (1950) reports the probable presence of appreciable amounts of saponins. Klein (1932) reports the presence of saponin "pittosporin", in the leaves.
(24)Corbett and McDowall (1958) have isolated the triterpenes, betulic acid, oleanolic acid, and ursolic acid acetate from the bark.
(25)Corbett and McGraw (1959) have isolated the triterpenes, betulic acid, ursolic acid acetate, and an unidentified triterpene acid from the bark.
(26)Cambie and Seelye (1961) have isolated the triterpenes, ursolic acid and betulic acid from the flowers.
(27)R. E. Corbett (pers. comm.) has reported the occurrence of the triterpene arjunolic acid in the bark.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
Metrosideros umbellata Cav.	southern rata	L	Herbarium	—	50406		—	+	—	—
		W, B	,,	—	,,		—	+	—	+
		S	,,	—	,,		—	+	—	—
Metrosideros parkinsonii Buchan.		L	Herbarium	—	26569		—	—	—	.
		W	,,	—	,,		—	+	—	—
		Fl	,,	—	,,		—	+	—	—
Metrosideros fulgens Sol. ex Gaertn.	aka-tawhiwhi	L	Herbarium	—	69468		—	+	—	—
		St, B	,,	—	,,		—	+	+	+
		Fl	,,	—	,,		—	+	—	—
Metrosideros perforata A. Rich.	aka-torotoro	L	Auckland	Aug.		—		+	—	
		W	,,	,,		—	—	+	—	—
Metrosideros albiflora Sol. ex Gaertn.	akatea	L	Herbarium	—	26570		—	+	—	—
		W	,,	—	,,		—	+	—	—
		Fl	,,	—	,,		—	+	—	—
Metrosideros diffusa Smith	akakura	L	Herbarium	—	15077		—	+	—	—
		T	,,	—	,,		—	+	—	—
		S	,,	—	,,		—	+	—	—
Metrosideros colensoi Hook. f.	hairy climbing rata	L	Herbarium	—	46130		—	+	—	—
		St	,,	—	,,		—	+	—	—
		Fl	,,	—	,,		—	+	—	—
Eugenia maire A. Cunn.	mairetawhake	L	Huia	March		—	—	—	—	+(28)
		W	,,	,,		—	—	—	—	+
		RW	,,	,,		—	—	—	—	+
		B	,,	,,		—	—	—	—	+
		RB	,,	,,		—	—	—	—	+
Lophomyrtus bullata Burret	ramarama	L	Auckland	Aug.		—	—	+	—	—
		W	,,	,,		—	—	+	—	+
Lophomyrtus obcordata Burret	rohutu	L	Auckland	Aug.		—	—	+	—	—
		W, B	,,	,,		—	—	+		

Neomyrtus pedunculata Allan	rohutu (pedunculate myrtle)	L	Stewart Is.	Jan.	44413	—	—	—	—	—
		W, B	,,	,,	,,	—	—	—	+	—
ELAEOCARPACEAE										
Elaeocarpus dentatus Vahl	hinau	L	Huia	March		—	—	+	—	—
		W	,,	,,		—	—	+	—	—
		RW	,,	,,		—	—	+	—	—
		B	,,	,,		—	—	+	—	—
		RB	,,	,,		—	—	+	—	—
Elaeocarpus hookerianus Raoul	pokaka	L	Ohakune	Oct.		—	—	—	—	—
		W	,,	,,		—	—	+	—	—
		R	,,	,,		—	—	—	—	—
Aristotelia serrata W. R. B. Oliver	makomako	L	Waitakeres	Feb.		+	+	—	—	—
		W	,,	,,		—	—	—	—	—
		B	,,	,,		—	—	+	—	—
		R	,,	,,		—	—			
Aristotelia fruticosa Hook. f.	mountain-currant	L	Herbarium	—	46544		—	—	—	
		W	,,	—	,,		—	+	—	—
TILIACEAE										
Entelea arborescens R. Br.	whau	L	Waikato Heads	Jan.		—	(+)	+[29]	—	—
		W	,,	,,		—	—	+	—	—
		RW	,,	,,		—	—	+	—	—
		B	,,	,,		—	(+)	—	—	—
		RB	,,	,,		—	—	—	—	—
		F	,,	,,		—	—	—	—	—
MALVACEAE										
Plagianthus betulinus A. Cunn.	manatu	L	Herbarium	—	58002				—	—
		W	,,	—	,,		—	—	—	—
		F	,,	—	,,		—	+	—	—

([28])Briggs, Cambie, Lowry and Seelye (1961) and Briggs and Cambie (in press) have reported the occurrence of the triterpenes, betulic acid in the bark and betulic and oleanolic acids in the leaves.

([29])Bate-Smith and Metcalfe (1957) record the presence of leucocyanidin in the leaves.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
Plagianthus divaricatus J. R. et G. Forst.	makaka	W	Auckland	April			—	—	—	—
		B	,,			—	—(30)	+	—	—
Hoheria populnea A. Cunn.	houhere	L	Waitakeres	March	46497		—	—	—	—
		W	,,	,,			—	—	—	—
		RW	,,	,,			—	—	—	—
		B	,,	,,			—	—	—	—
		RB	,,	,,			—	—	—	—
		Fl	,,	,,			—	—	—	—
Hoheria sexstylosa Col.	long leaved lacebark	L, St, Fl	Herbarium	—	240		—	—(31)	—	—
Hoheria angustifolia Raoul	narrow-leaved lacebark	L	Auckland	Aug.		—	—	—	—	—
		St	,,	,,		—	—	—	—	—
Hoheria glabrata Sprague et Summerhayes		L, W, B, Fl	Herbarium	—	44121		—	—	—	—
Hibiscus trionum L.	starry hibiscus	H	Gt. Barrier Is.	Sept.		—	—	+	—	—
Hibiscus diversifolius Jacquin		L	Auckland	Aug.		—	—	+	—	—
		W	,,	,,		—	—	+	—	—
		B	,,	,,		—	—	+	—	—
EUPHORBIACEAE										
Homalanthus polyandrus Cheesem.		W	Kermadec Is.	June		—	—	—	—	—
		B	,,	,,		—	—	—	—	—
Euphorbia glauca Forst. f.	waiuatua (Maori splurge)	L, St	Herbarium	—	50350		—	—	—	—
CUNONIACEAE										
Ackama rosaefolia A. Cunn.	makamaka	L	Auckland	Aug.		—	—	+	—	—
		W				—	—	+	—	—

Weinmannia silvicola Sol. ex A. Cunn.	tawhero	L	Kaimai ranges	May		–	–	+	–	–
		W	,,	,,		–	–	+	+	–
		RW	,,	,,		–	–	+	+	(+)
		B	,,	,,		–	–	+	+	–
Weinmannia racemosa Linn. f.	kamahi	L	Ohakune	Oct.	50071	–	–	+	–	–
		W	,,	,,		–	–	+	–	–
		B	,,	,,		–	–	+	–	–
ESCALLONIACEAE										
Quintinia serrata A. Cunn.	tawhewheo	L	Waitakeres	March	46469	–	–	+	–	–
		W	,,	,,		–	–	+	–	–
		RW	,,	,,		–	–	+	–	–
		B	,,	,,		–	–	+	–	–
Quintinia acutifolia Kirk	mountain tawhewheo	L	Herbarium	—	28048		–	+	+	–
		W, B	,,	—			–	+	+	–
Ixerba brexioides A. Cunn.	tawari	L	Waitakeres	April		–	–	–	–	–
		W	,,	,,		–	–	–	–	–
		B	,,	,,		–	–	+	–	–
Carpodetus serratus J. R. et G. Forst.	putaputaweta	L	Auckland	April			–	–(32)	–	+
		W	,,	,,			–	–	–	++
		B	,,	,,			+	–	–	+
ROSACEAE										
Rubus schmidelioides A. Cunn.	swamp-lawyer	L	Waitakeres	Sept.		–	–	–	+	–
		W	,,	,,		–	–	+	+	–
		RW	,,	March		–	–	+	+	–
		B	,,	Sept.		–	–	+	+	–
Rubus australis Forst. f.	tataramoa	L	Waitakeres	March	46454	–	–	–	+	–
		W	,,	,,		–	–	+	+	–
		B	,,	,,		–	–	+	+	–
		RB	,,	,,		–	–	+	+	–
		L	Ohakune	Dec.	50083		–		+	–

(30)Anomalous test. Dragendorff's reagent gave a black precipitate and Mayer's reagent gave a pink colour changing to blue but no precipitate.
(31)Bate-Smith and Metcalfe (1957) record the presence of leucocyanidin in the leaves. This has not been confirmed with the present sample.
(32)Bate-Smith and Metcalfe (1957) record the possible presence of leucodelphinidin in the leaves. This has not been confirmed in the present work.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
Rubus cissoides A. Cunn.	tataramoa (bush-lawyer)	L	Waitakeres	Oct.		—	—	—	—	—
		St	,,	,,		—	—	—	—	—
		Fl	,,	,,		—		—	—	
Acaena microphylla Hook. f.	small-leaved acaena	L, St	National Park	March		—			—	
		Fl	,,	,,		—	—	+	—	—
Acaena anserinifolia Druce	piripiri (biddy biddy)	H	Maungatepopo	Nov.	50103	—	—	+	+	—
Potentilla anserinoides Raoul	silver-weed	L, St	Waikaremoana	Nov.		—	—	+	—	—
Geum leiospermum Petrie		H	Herbarium	—	50034		—	+	—	—
Geum parviflorum Smith	avens	H	Herbarium	—	32480		—	+	—	
Geum urbanum L. var. *strictum* Hook. f.	kopata (common avens)	H	Herbarium		70618		—	+(33)	—	—
PAPILIONACEAE										
Canavalia obtusifolia DC.		L	Herbarium	—	44112	—	(+)(34)	—	—	—
Swainsona novae-zelandiae Hook. f.		L, St, Fl	Herbarium	—	15792	+	+	—	—	—
Clianthus puniceus Sol. ex Lindl.	parrots bill (kaka beak)	L	Auckland	Aug.		+	+(35)		—	
Sophora prostrata Buchan.	dwarf kowhai	L	Auckland	Sept.		++	+	—	—	—
Sophora tetraptera J. Mill.	kowhai	L	Kawakawa	Jan.		+++	+++(36)	—	—	—
		RW	,,	,,		++	+++	—		—
		RB	,,	,,		+	+	—	—	—
		S	,,	,,		++	+++	—	—	—
Sophora microphylla Ait.	kowhai	L	Auckland	Sept.		+	+	—		—
		B	,,	,,		++	+++(37)	—	—	—
		Fl	,,	,,		+++	+++	—	—	
		S	,,	,,		+++	+++	—	—	—
Corallospartium crassicaule J. B. Armst.	coral bloom	H	Herbarium	—	24233	+	+(38)	—		—

Notospartium carmichaeliae Hook. f.		L, St	Herbarium	—	342	+	+[38]	—	(+)	—
Chordospartium stevensonii Cheesem.		H	Herbarium	—	49484	+	+[38]	—	—	—
Carmichaelia grandiflora Hook. f.	large flowered broom	H	Herbarium	—	65429	—	—[38]	—	—	
Carmichaelia glabrata Simpson		H	Herbarium	—	46055	—	—[38]	—	—	
Carmichaelia odorata Col. ex Hook. f.	sweet-broom	H	Auckland	Aug.		(+)	+[38]	—	+	—
Carmichaelia williamsii Kirk		H	Auckland	Aug.		(+)	+[39]	—	—	
Carmichaelia aligera Simpson	makaka	L	Waitakeres	March		+	++	—	—	—
		W	,,	,,		—	—	—	—	—
		B	,,	,,		—	—	—	—	—
		RB	,,	,,		—	—	—	—	—
		S	,,	Feb.		—	—	—	—	—
Carmichaelia cunninghamii Raoul		H	Herbarium	—	22307	+	+[40]	—	—	—
Carmichaelia egmontiana Simpson		H	Herbarium	—	70619	—	—[40]	—	—	
Carmichaelia flagelliformis Col. ex Hook. f.	maukoro	H	Herbarium	—	61963	—	+[40]		—	—
Carmichaelia robusta Kirk		H	Herbarium	—	70626	+	+	—		—
Carmichaelia appressa Simpson		H	Herbarium	—	70625	+	+	—	—	—
Carmichaelia rivulata Simpson	Simpson	H	Herbarium	—	70624	—	—[40]	—		—
Carmichaelia violacea Kirk		L	Auckland	Sept.		+	+	—	—	
Carmichaelia petriei Kirk		H	Herbarium	—	70621	+	+[40]	—	—	—
Carmichaelia petriei Kirk var. *minor* Simpson		H	Herbarium	—	70620	—	+	—	—	—

([33])Bate-Smith and Metcalfe (1957) record the absence of leucoanthocyanins in the leaves of *Geum urbanum*.

([34])Webb (1952) records positive alkaloid tests for the mature seeds.

([35])White (1943, 1951, 1957) reports these species to be "alkaloid free". White states that simple bases, betaines, or phenolic bases would not necessarily have been detected in his tests.

([36])Briggs and Taylor (1938) report the occurrence in the seeds of the alkaloids matrine, methylcytisine, and sophochrysine.

([37])Briggs and Ricketts (1937) report the occurrence of the alkaloids matrine, cytisine, methylcytisine, sophochrysine and a further unidentified alkaloid in the seeds while Briggs, Cambie, Holdgate, and Seelye (1960) have reported the occurrence of the same alkaloids in the bark and flowers and the additional presence of anagyrine in the flowers.

([38])White (1943, 1951, 1957) reports these species to be "alkaloid free". White states that simple bases, betaines, or phenolic bases would not necessarily have been detected in his tests.

([39])White (1943, 1951, 1957) reports these species to be "alkaloid free". White states that simple bases, betaines, or phenolic bases would not necessarily have been detected in his tests.

([40])White (1943, 1951, 1957) reports these species to be "alkaloid free". White states that simple bases, betaines, or phenolic bases would not necessarily have been detected in his tests.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
Carmichaelia ramosa Simpson		H	Herbarium	—	70628	+	+	—	—	—
Carmichaelia virgata Kirk		H	Herbarium	—	70622	—	—	—		
Carmichaelia kirkii Hook. f.		H	Herbarium	—	70622a	+	+(40)	—	—	—
Carmichaelia orbiculata Col.		L, W, B	Herbarium	—	50822		—(41)	—	—	—
Carmichaelia corrugata Col.	dwarf broom	H	Herbarium	—	70626a	+	+(41)	—	—	—
Carmichaelia uniflora Kirk		H	Herbarium	—	1935	+	+(41)	—	—	—
Carmichaelia astonii Simpson		L, Fl, S	Herbarium	—	70629	+	+(41)	—	(+)	—
Carmichaelia monroi Hook. f.	stout dwarf broom	L, S	Herbarium	—	323	+	+(41)	—	—	—
		L	Auckland	Sept.		++	+++	—	—	—
Carmichaelia compacta Petrie		L, St	Herbarium	—	232	+	+(41)	—	(+)	—
FAGACEAE										
Nothofagus menziesii Oerst.	tawai (silver beech)	L	Auckland	Aug.		—		—	—	
		W	,,	,,		—	—	—	—	+
Nothofagus fusca Oerst.	tawai (red beech)	L	Silverdale	July		—	—	+	—	—
		B	,,	,,		—	—	+	—	—
Nothofagus truncata Ckn.	hard-beech	L	Albany	June	50330	—	—	+	—	—
		W, B	,,	,,		—	—	+	—	—
Nothofagus solandri Oerst.	black-beech	L, W, B	Herbarium	—	70630	—	-	+	—	-
		L	Auckland	Sept.		—	—	+	—	—
Nothofagus solandri Oerst. var. *cliffortioides* Oerst.	mountain-beech	L	Ohakune	Jan.	50896	—	—	+	—	—
		W	,,	,,		—	—	+	—	—
		B	,,	,,		—	—	+	+	—
		RB	National Park	Oct.	46548	—	-	+	—	
MORACEAE										
Paratrophis smithii Cheesem.	Three Kings milk tree	L	Herbarium	—	44827		—	—	—	—
		W, B	,,	—	,,		—	—	—	—
		S	,,	—	,,		—			

Paratrophis banksii Cheesem.	large-leaved milk-tree	L	Herbarium	—	44017		—	—	—	—
		W, B	,,	—			—	—	—	—
		S	,,	—			—	—	—	—
		L	Auckland	Sept.		—	—	—	—	—
Paratrophis microphylla Ckn.	milk-tree	L	Auckland	Aug.		+	—	—	—	—
		W	,,	,,		+	—		—	—
URTICACEAE										
Boehmeria dealbata Cheesem.		L	Kermadec Is.	June	44145		—	+	—	—
		W, B	,,	,,	,,		—	+	—	—
Urtica ferrox Forst. f.	ongaonga (tree-nettle)	L	Auckland	Aug.		—	—	—	—	—
		W	,,	,,		—	—	—	—	—
Urtica incisa Poir.	dwarf nettle	L	Herbarium	—	44813			—	—	—
Elatostema rugosum A. Cunn.	parataniwha	L	Waitakeres	May		—		—	—	—
Parietaria debilis Forst. f.	pellitory	H	Huia	Oct.			—	—	—	—
Australina pusilla Gaud.		H	Herbarium	—	58855			—	—	—
CORYNOCARPACEAE										
Corynocarpus laevigatus J. R. et G. Forst.	karaka	L	Auckland	Dec.		—	—	—([41])	—	—
		W	,,	,,		+++	+++	—	+	—
		RW	,,	,,		++	++	—	+	—
		B	,,	,,		—		—	—	—
		RB	,,	,,			—	—	—	—
		F	,,	,,		+	+		—	—
ICACINACEAE										
Pennantia corymbosa J. R. et G. Forst.	kaikomako	L	Auckland	Aug.		—	—	+	—	—
		W	,,	,,				+	—	—
STACKHOUSIACEAE										
Stackhousia minima Hook. f.		H	Herbarium	—	2082		—	+	(+)	—

([41])Bate-Smith and Metcalfe (1957) record the presence of leucocyanidin in the leaves. This has not been confirmed in the present work.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
SANTALACEAE										
Exocarpus bidwillii Hook. f.		L	Auckland	Sept.		—	—	+	—	(+)
Mida solicifolio A. Cunn.	taiko	L	Auckland	Sept.		—	—	—	—	—
LORANTHACEAE										
Kortholsella salicornioides Tiegh.		L, W	Auckland	Aug.		—	—	+	—	+
		S	(Cult.)	,,		—	—	+	—	+
Loranthus micranthus Hook. f.		L, T	Herbarium	—	69471		—	+	—	—
		L	Auckland	Sept.		—	—	+	—	—
Tupeia antarctica Cham. et Schlecht.	pirita	L	Herbarium	—	14758		—	—	—	—
		W, B	,,	—	,,		—	—		—
Elytranthe colensoi Engl.	korukoru (scarlet mistletoe)	L	Herbarium	—	40181		—	+	—	—
		W, B	,,	—	,,		—	+	—	—
		Fl	,,	—	,,		—	+	—	—
Elytranthe tetrapetala Engl.	pirirangi	L, Fl	Ohakune	Jan.	44418	+	+	+	—	
		W	,,	,,		+	++	+	—	—
		W, B	,,	,,		(+)	(+)	+	—	—
		F	Rotorua	May	46515	—	—	+	—	—
Elytranthe adamsii Engl.		L	Herbarium	—	70631		—	+	—	—
		W, B	,,	—	,,		—	+	—	—
Elytranthe flavida Engl.	yellow mistletoe	L	Herbarium	—	32486		—	+	—	—
		W, B	,,	—	,,		—	+	—	—
BALANOPHORACEAE										
Dactylanthus taylori Hook. f.	puareinga	Rh	Herbarium	—	28729		—	—		

RHAMNACEAE										
Discaria toumatou Raoul	tumatukuru	L, T	Ashburton	Dec.		–	–	–	+	–
		W	,,	,,		–	–	–	+	–
		RB	,,	,,		–		+	+	–
Pomaderris kumeraho A. Cunn.	kumarahou	L	Waitakeres	Feb.	46478	–	–	+	+(42)	–
		W	,,	,,		–	–	+	+	–
		RW	,,	March		–	–	+	+	–
		B	,,	,,		–	–	+	+	–
Pomaderris apetala Labill	tainui	L	Auckland	Sept.		–	–	+(43)	+	+
		W	,,	,,		–		+	+	–
		S	,,	,,		–				
Pomaderris prunifolia A. Cunn. ex Fenzl var. *edgerleyi* L. B. Moore	kumarahou	L	Auckland	Sept.	50857	–	–	+	–	+
Pomaderris rugosa Cheesem.		L	Waitakeres	March	46484	–	–	+	–	–
		W	(cult.)	,,		–	–	+	+	–
		B	,,	,,		–	–	+	+	–
		R	,,	,,		–	–	+	+	–
Pomaderris phylicifolia Lodd. var. *ericifolia* L. B. Moore	tauhinu	L, S	Taupo	Oct.	46928	–	–	+	+	–
		RW	,,	,,	,,	–	–	+	+	
RUTACEAE										
Phebalium nudum Hook.	mairehau	L	Waitakeres	March		+	+	–	–	–
		W	,,	,,		+	+	–	–	–
		RW	,,	,,		+	+	–	–	–
		B	,,	,,		+++	+++(44)	–	–	
		RB	,,	,,		+	+	–	–	
Melicope ternata J. R. et G. Forst.	wharangi	L, T	Waitakeres	Jan.		+	++	–		
		W	,,	,,		++	+++	–	–	–
		B	,,	,,		++	+++(45)	–		

(42)Murray (1950) reports the probable presence of appreciable amounts of saponins in *Pomaderris* species. Briggs (1947) and Cain and Cambie (1959) report the presence of saponins in *Pomaderris kumeraho* (=*P. elliptica*).

(43)Bate-Smith and Metcalfe (1957) record the presence of leucocyanidin in the leaves.

(44)Briggs and Cambie (1958a) have shown the presence of the furoquinoline alkaloids, dictamnine, γ-fagarine, evolitrine, skimmianine, and kokusaginine in the bark and Cambie (1959c) has shown the presence of dictamnine, skimmianine, kokusaginine, and an unidentified alkaloid in the wood.

(45)Briggs and Locker (1949) have isolated the alkylated flavonols, meliternatin, meliternin, and ternatin from the bark. These give precipitates with the usual alkaloid reagents and alkaloids themselves are possibly absent in the plant.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
Melicope ternata J. R. et G. Forst. var. *mantelli* Buch.		L	Auckland	Feb.		+	++	–	–	–
		W	,,	,,		++	++	–	–	–
		B	,,	,,		++	++(46)	–	–	–
Melicope simplex A. Cunn.	poataniwha	W	Waitakeres	April	46500	–	–	–	–	–
		B	,,	,,		–	–(47)	–	–	.
		R	,,	,,		–	–	–	–	.
MELIACEAE										
Dysoxylum spectabile Hook. f.	kohekohe	L	Waitakeres	May		–	–	–	–	–
		W	,,	,,		–	–(48)	+(48)	–	.
		RW	,,	,,		–	–	+	–	.
		B	,,	,,		–	–(48)	+(48)	–	–
		F	,,	Sept.		–	–	–	–	+
		L	Herbarium	—	44774 &		–	–	–	–
		T	,,	—	50701		–	+	–	–
		Fl, S	,,	—	,,		–	+	–	–
ANACARDIACEAE										
Plectomirtha baylisiana		L	Herbarium	—	22875		–	–		–
W. R. B. Oliver		T	,,	—	,,		–	(+)	–	.
		S	,,	—	,,		–	–	–	.
SAPINDACEAE										
Alectryon excelsus Gaertn.	titoki	L	Waitakeres	March		–	–	+(49)	–	–
		W	,,	,,		–	–	+	(+)	–
		RW	,,	,,		–	–	+	–	–
		B	,,	,,		–	–	+	(+)	–
		RB	,,	,,		–	–	+	–	–
Dodonaea viscosa Jacquin	akeake	L	Waitakeres	March			–(50)	+(49)	–	–
		W	,,	,,			–	+	+(51)	–
		B	,,	,,			–	+	–	–
		RB	,,	,,			–	+	–	–
		L	Auckland	Sept.			–	+	–	–
		W	,,	,,			–	+	+	+

ARALIACEAE										
Kirkophytum lyallii Allan	punui	L	Herbarium	—	764		—	—	+	—
Meryta sinclairii Seem.	puka	L	Auckland	June		—	—	—	—	—
		W	,,	,,			—	—	—	
		B	,,	,,		—	—	—	—	
Schefflera digitata J. R. et G. Forst.	pate (five-finger)	L	Waitakeres	May		—	—	—	+	
		RW	,,	,,		+	+ +	—	+	—
		B	,,	,,			—		+	
		RB	,,	,,		—	—		+	
Neopanax anomalum Allan	wawaupaka	L, T	Ohakune	Oct.	50073	—	—			+
		W	,,	,,		—		—	—	+ +
		B	,,	,,		—	—	—	—	+
Neopanax simplex Allan	haumakoroa	L	National Park	Nov.	50139	—		—	+	+
		W, B	,,	,,				—	—	+
Neopanax simplex Allan var. *sinclairii* Allan	mountain panax	L	National Park	Nov.	50108			—	—	+
		W	,,	,,			—	—	—	+
		B	,,	,,				—	—	+
Neopanax colensoi Allan	orihou	L	National Park	Nov.	50106			—	+	+
		B	,,	,,		—	—	—	—	—
		W	,,	,,		—	—	—	+	+
		S	,,	,,		—	—	—	+	+
Neopanax arboreum Allan	puahou	L	Kawakawa	Jan.		—	—	—	—	—
		W	,,	,,		—			+	
		RW	,,	,,		—	—		—	
		B	,,	,,		+	+	—	+	
		RB	,,	,,		+	+	—	—	—
		F	Waitakeres	Aug.		(+)	(+)	—	+	+

(46)Cambie (1960) has isolated the same flavonols from the bark as are present in *M. ternata*.

(47)Briggs and Locker (1950) record that alkaloids are not present in appreciable quantity.

(48)Cambie (1959a) records the absence of alkaloids and leucoanthocyanins in the bark and heartwood. Leucoanthocyanins have now been shown to be present.

(49)Bate-Smith and Metcalfe (1957) record the presence of leucocyanidin in the leaves.

(50)Webb (1949) reports positive alkaloid tests for the leaves.

(51)Murray (1950) reports the probable presence of appreciable amounts of saponins.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
Neopanax laetum Allan		W	Huia	March		—	—	—	+	-
		RW	,,	,,		—	—	—	+	—
		B	,,	,,		—	—	—	—	+
		RB	,,	,,		—	—	—	—	+
Pseudopanax edgerleyi C. Koch	raukawa	L	Waitakeres	May	44407	—	—	—	—	—
		W	,,	,,		—	—	—	—	-
		B	,,	,,		—	—	—	—	+
Pseudopanax lineare C. Koch	narrow-leaved panax	L	Herbarium	—	32602		—	—	+(52)	—
		T	,,	—	,,		—	—	+	—
Pseudopanax crassifolium C. Koch	horoeka (lance-wood)	L	Waitakeres	March	46466	—	—	—	+	+
		W	,,	,,		—	—	—	—	+
		RW	,,	,,		—	—	—	—	+
		B	,,	,,		—	—	—	+	+
Pseudopanax crassifolium C. Koch var. *unifoliolatum* Kirk		L	Karekare	March	46474	—	—	—	—	+
Pseudopanax ferox Kirk	toothed lancewood	L	Auckland	Sept.		—	—	—	+	—
Pseudopanax chathamicum Kirk	Chatham lancewood (houhou)	L	Herbarium	—	2303		—	—	+	—
		T	,,	—	,,		—	—	+	—
		S	,,	—	,,		—	—	—	—
Pseudopanax lessonii C. Koch	houpara	L	Auckland	Sept.		—	—	—	+	—
		W	,,	,,		—	—	—	+	—
Pseudopanax discolor Harms	bronze panax	L	Auckland	Sept.		—	—	—	+	—
		W	,,	,,		—	—	—	+	+
		L	Herbarium	—	28011		—	—	+	—
		W	,,	—	,,		—	—	+	+
CORNACEAE										
Corokia cotoneaster Raoul	mountain korokio	L	Auckland	Sept.		—	—	+	—	+
		W	,,	,,		—	—	+	—	—
		F	,,	,,		—	—	+	—	—

Corokia buddleioides A. Cunn.	korokio-taranga	L	Waitakeres	March		–	–	+	–	–
		W	,,	,,		–	–	+	+	–
		RW, RB	,,	,,		–	–	+	–	–
		B	,,	,,		–	–	+	–	–
Corokia macrocarpa Kirk	hokataka	L	Herbarium	—	2256		–	–	–	–
		T	,,	—	,,		–	+	–	–
Griselinia lucida Forst. f.	puka	L	Waitakeres	March		–	–	–	(+)	–
		W	,,	,,		–	–	–	–	–
		RW	,,	,,		–	–	–	–	–
		B	Stewart Is.	Jan.		–	–	–	–	–
		RB	Waitakares	March		–	–	–	–	–
		L	Herbarium	—	31847		–	–	(+)	–
		T	,,	—	,,		–	–	–	–
Griselinia littoralis Raoul	papauma	R	Waitakeres	April	,,	–	–	–([53])	–	–
UMBELLIFERAE										
Hydrocotyle dissecta Hook. f.	cut-leaved marsh penny-wort	H	Herbarium	—	50869	–	–	–	–	–
Apium australe Thouars	Maori celery	H	Piha	May			–([54])	–	–	–
Aciphylla squarrosa J. R. et G. Forst.	taramea	L	Auckland	Sept.		–	–	–	–	–
Aciphylla scott-thomsonii Ckn. et Allan	greater-spaniard	S	Cardrona Valley	Jan.	44397	+	++	–	–	
Anistome aromatica Hook. f.	kopoti	R	National Park	Nov.	50123	–	–	–	–	–
Angelica montana Ckn.	Maori anise	L, St	Auckland	Aug.		–	-	–	–	-
Angelica rosaefolia Hook.	koheriki	L	Piha	Aug.		–	–	-	–	(+)
		St	,,	,,		-	-	–	–	–
		L	Herbarium	—	58009	–	-	–	–	(+)
		St	,,	—	,,	–		–	–	–
		Fl	,,	—	,,	+	+	–	–	(+)

([52])Murray (1950) reports the probable presence of appreciable amounts of saponins in *Pseudopanax* species.

([53])Bate-Smith and Metcalfe (1957) record the absence of leucoanthocyanins in the leaves.

([54])Webb (1952) records negative alkaloid tests.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
ERICACEAE										
Gaultheria antipoda Forst. f.	taupuku	L	Maungatepopo	Nov.	46520	—	—	+	—	+
		W	,,	,,		—		+	—	+
Gaultheria depressa Hook. f.	mountain-snowberry	L	National Park	Oct.	46546	-	—	+	—	—
		T	,,	,,	,,	—	—	+	—	—
Gaultheria colensoi Hook. f.		L	Herbarium	—	70102		—	+	-	+
Gaultheria rupestris D. Don	vale-lily snowberry	L	Herbarium	—	58876		—	+	-	+
Gaultheria oppositifolia Hook. f.	niniwa	L	Herbarium	—	2354		—	+	(+)	+
		W	,,	—	,,		—	+	—	—
		S	,,	—	,,		—	+	(+)	+
Pernettya nana Col.		H	Herbarium	—	70103		—	+	-	-
Pernettya macrostigma Col.		L, T	Maungatepopo	Nov.	50142	—	—	+	—	+
EPACRIDACEAE										
Pentachondra pumila R. Br.	little-mountain heath	H	Waiouru	Oct.	50065 & 50137	—	—	+	+	—
Cyathodes fasciculata Allan	mingimingi	L	Waitakeres	March		—	—	+	+	+
		W	,,	April		—	—	+	+	—
		B	,,	March		—	—	+	—	—
Cyathodes parviflora Allan		L, T, Fl	Herbarium	—	50805			+	-	—
Cyathodes juniperina Druce	taumingi	L	Waitakeres	March	50056	—	—	+	+	—
Cyathodes colensoi Hook. f.		L, T	Herbarium	—	44398		—	+	—	—
Cyathodes fraseri Allan	totarapapa (dwarf heath)	L, T	Waitakeres	Nov.		—	—	+	—	—
Cyathodes empetrifolia Hook. f.		L, T	National Park	Nov.	50119	—	—	+	+	
		W	,,	,,		—	—	+	+	—
Cyathodes pumila Hook. f.		L	Herbarium	—	70104		—	+	—	—

Archeria racemosa Hook. f.		L	Herbarium	—	28017		—	+	—	+
Archeria traversii Hook. f.		L, T	Herbarium	—	32424		—	+	—	+
Epacris pauciflora A. Rich.	tamingi	L, St	Herbarium	—	24427		—	+	—	+
Epacris alpina Hook. f.	nehenehe	L	Herbarium	—	50061		—	+	—	+
		T	,,	—	,,		—	+	—	—
Dracophyllum muscoides Hook. f.		H	Herbarium	—	70106	—	—	+	—	
Dracophyllum pronum W. R. B. Oliver		L	Auckland (Cult.)	Sept.		—	—	+	—	+
Dracophyllum politum Ckn.	cushion-forming heath	L	Herbarium	—	44162	—	—	+	—	—
Dracophyllum pearsonii Kirk	Pearson's needle-leaved heath	L	Herbarium	—	11572	—	—	+	—	—
Dracophyllum rosmarinifolium R. Br.	prostrate needle-leaved heath	L	Auckland	Sept.		—	—	+	—	+
		W, B	(Cult.)	,,		—	—	+	—	+
Dracophyllum uniflorum Hook. f.	one-flowered grass-tree	L	Auckland	Sept.		—	—	+	—	+
		W, B	(Cult.)	,,		—	—	+	+	+
Dracophyllum subulatum Hook. f.	monoao	L	Herbarium	—	46532	—	—	+	—	—
		W	,,	—	,,	—	—	+	+	—
		B	,,	—	,,	—		+	+	—
Dracophyllum urvilleanum A. Rich.	wharekohu	L, S	Herbarium	—		—	—	+	—	—
		W		—		—	—	+	—	—
Dracophyllum filifolium Hook. f.		L	National Park	May			—	+	—	+
		W, B	,,	,,		—	—	+	+	+
Dracophyllum longifolium R. Br.	inanga	L, T	Herbarium	—	70107	—	—	+	—	—
Dracophyllum sinclairii Cheesem.		L	Herbarium	—	32648	—	—	+	—	—
Dracophyllum patens W. R. B. Oliver		L	Herbarium	—	40213	—	—	+	—	—
Dracophyllum viride W. R. B. Oliver		L	Herbarium	—	1695	—	—	+	—	—
Dracophyllum recurvum Hook. f.	red heath	L	Ohakune	Jan.		—	—	+	—	—
		W, B	,,	,,		—	—	+	—	—
		RW	,,	,,		—	—	+	—	—
Dracophyllum menziesii Hook. f.		L	Herbarium	—	25808	—	—	+	—	—
Dracophyllum townsonii Cheesem.		L	Herbarium	—	515	—	—	+	—	—

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
Dracophyllum strictum Hook. f.	totorowhiti	L	Herbarium	—		—	—	+	—	—
		St		—		—	—	+	—	—
		Fl		—		—	—	+	—	—
Dracophyllum latifolium A. Cunn.	neinei (spider wood)	L	Waitakeres	March		—	—	+	—	—
		St	,,	,,		—	—	+	—	—
		RW, RB	,,	,,		—	—		—	—
		B	,,	,,		—	—	+	—	—
Dracophyllum traversii Hook. f.	mountain neinei	L	Herbarium	—	50971	—	—	+	—	—
SAPOTACEAE										
Planchonella novo-zelandica Allan	tawapou	L	Karekare	March	46468	+	+	—	+	—
		W	,,	,,		—	—	—	+	—
		RW	,,	,,		—	—	—	+	—
		B	,,	,,		—	—	+	+	—
		RB	,,	,,		—	—	+	+	—
MYRSINACEAE										
Myrsine kermadecensis Cheesem.		L, S	Kermadec Is.	June	44200	—	—	+	—	(+)
		B	,,	Feb.	44144	—	—	+	—	+
Myrsine salicina Hew ex Hook. f.	toro	L	Waitakeres	Sept.		—	—	+	+	+
		W, B	,,	,,		—	—	+	+	+
Myrsine australis Allan	mapau (red matipo)	L	Waitakeres	March		—	—	+	+	—
		W	,,	,,		—	—	+	+	—
		B	,,	,,		—	—	+	+	—
		RB	,,	,,		—	—	+	+	—
		Fl	,,	Aug.		—	—	+	—	—
Myrsine chathamica F. Muell.	Chatham matipo	L	Herbarium	—	70631	—	—	+	+	+
		T	,,	—	,,	—	—	+	+	+

Myrsine divaricata A. Cunn.	weeping matipo	L	Auckland	Sept.		−	−	+	−	−
		W, B	,,	,,		−	−	+	(+)	−
Myrsine nummularia Hook. f.	creeping matipo	L	Auckland	Sept.		−	−	+	−	−
OLEACEAE										
Gymnelaea apetala Vahl.	broad-leaved maire	L	Herbarium	—	23287		−	−	−	+
Gymnelaea cunninghamii Hook. f.	maire	L	Ohakune	Oct.	50098	−	(+)	−	−	−
		W	,,	,,		+	+	−	−	−
		B	,,	,,		+	+	−	−	−
		RB	,,	,,		+	+	−	−	−
Gymnelaea lanceolata Hook. f.	white maire	L	Auckland	Aug.		−	−	−	−	−
		W	,,	,,		−	−	−	−	−
Gymnelaea montana Hook. f.	rororo	L	Huia	March	46494	+	+	−	−	−
		RW	,,	,,		−	−	−	−	−
		B	,,	,,		−	−	−	−	−
		RB	,,	,,		−	−	−	−	−
LOGANIACEAE										
Geniostoma ligustrifolium A. Cunn.	hangehange	L	Waitakeres	March		−	−	−	−	+
		W	,,	,,		−	−	−	−	−
		RW	,,	,,		−	−	−	−	+
		B	,,	,,		−	−	−	−	−
		RB	,,	,,		−	−	−	−	−
APOCYNACEAE										
Parsonsia heterophylla A. Cunn.	kaihua	L	Waitakeres	Jan.		−	+	−	−	−
		W	,,	Nov.		++	++	−	−	−
		RW	,,	,,		+	+	−	−	−
		B	,,	,,		+	+	−	−	−
		RB	,,	,,		+	++	−	−	−
		Fl	,,	Jan.		−	+	−	−	−
		S	,,	,,		−	+	−	−	−
Parsonsia capsularis R. Br.	akakiore	H	Waitakeres	Nov.	46501	++	++(55)	−	−	−

(55)E. P. White (pers. comm.) has shown the occurrence of alkaloids (0.06%) in the stems and leaves.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
CAPRIFOLIACEAE										
Alseuosmia macrophylla A. Cunn.	karapapa	W	Huia	March		—	—	—	—	—
		RW	,,	,,		—	—	—	—	—
		B	,,	,,		—	—	—	+	—
		RB	,,	,,		—	—	—	—	—
Alseuosmia quercifolia A. Cunn.	toropapa	L	Ohakune	Nov.	50090	—	—(56)	+	—	—
		T	,,	,,		—	—(56)	+	+	—
		B	,,	,,		—	—(56)	+	+	—
		RB, RW	,,	,,		—	—(56)	+	+	—
Alseuosmia linariifolia A. Cunn.	matukuroimata	L	Herbarium	—	40174	—	—	+	—	+
Alseuosmia banksii A. Cunn.	pere	L	Herbarium	—	36993	—	—	+	—	—
RUBIACEAE										
Coprosma pumila Hook. f.		H	National Park	Nov.	50134		—	—(57)	—	+(58)
Coprosma petriei Cheesem.	Petrie's coprosma	H	Herbarium	—	24960	—	—	—	—	(+)
Coprosma acerosa A. Cunn.	tarakupenga	B	Auckland	Aug.		—	—	—	—	+
Coprosma brunnea Ckn. ex Cheesem.		L	Auckland	Sept.		—	—	—	—	+
		St	,,	,,		—	—	—	—	—
		R	,,	,,		—	—		—	—
Coprosma rugosa Cheesem.	heath-like coprosma	L, St	Auckland	Sept.		—	—	—	—	+
Coprosma antipoda W. R. B. Oliver		L	Auckland	Aug.		—	—	—	—	+
		W, B	,,	,,		—	—	—	—	+
Coprosma cheesemanii W. R. B. Oliver		L	Herbarium	—	36905	—	—	—	—	+
		B	,,	—	,,	—	—	—	—	+
Coprosma depressa Col. ex Hook. f.	prostrate coprosma	L	Herbarium	—	36872	—	—	—	—	—

Coprosma pseudocuneata W. R. B. Oliver	wedge-leaved coprosma	L, T	National Park	Nov.	50125	–	–	–	–	+
		St, R	,,	,,		–	–		–	+
		B	,,	,,		–	–	–		+
Coprosma linariifolia Hook. f.	mikimiki	B	Herbarium	—	32542			–	–	+
Coprosma microcarpa Hook. f.	small-fruited coprosma	L, T	Taupo	Jan.	50062	–	–		–	+
Coprosma parviflora Hook. f.	leafy coprosma	B	Auckland	Sept.				–		+
Coprosma propinqua A. Cunn.	mingi	L, T	Herbarium	—	36755		–	–	–	+
Coprosma banksii Petrie	Bank's coprosma	L	Herbarium	—	58253		–		–	+
		St	,,	—	,,		–	–		+
Coprosma colensoi Hook. f.	Colenso's coprosma	L	Herbarium	—	58249	–		–		–
Coprosma foetidissima J. R. et G. Forst.	hupiro	L	National Park	Nov.		–	–	–	-	+
		W	,,	,,					–	+
		B	Stewart Is.	Jan.		–	–		–	+
Coprosma rubra Petrie		B	Auckland	Sept.		–	–	–	–	+
Coprosma rigida Cheesem.		L, T, B	Herbarium	—	61990				–	+
Coprosma crassifolia Col.	stiff-stemmed coprosma	L, T	Herbarium	—	44778			–	–	+
Coprosma rotundifolia A. Cunn.	round-leaved coprosma	T	Auckland	Sept.		–	–	–	+	–
Coprosma tenuicaulis Hook. f.	hukihuki	L, T	Ohakune	Oct.	50072	–	–	–	-	+
		W	,,	,,		–	–	–	–	+
		R	,,	,,		–	–	–	-	+
Coprosma areolata Cheesem.	areolate coprosma	W	Auckland	Jan.		–				+
		B	,,	,,		–			+	+
Coprosma spathulata A. Cunn.		L, T	Herbarium	—	37390			–	–	+
Coprosma arborea Kirk	mamangi	L	Waitakeres	Jan.	40224	–	–	–	–	+

([56])Dragendorff's reagent gave a purple colour but no precipitate.

([57])Briggs and Nichols (1954) have shown the presence of asperuloside in species of the Rubiaceae by a characteristic bluish-green colour on boiling with 2N-hydrochloric acid. The characteristic colour is also given in the test for leucoanthocyanins and the presence of asperuloside was confirmed in all species of the Rubiaceae examined.

([58])Anthraquinones shown to be present in species of the Rubiaceae (Briggs and co-workers, 1948, 1949, 1952, 1955; Brooker 1959) show strong colorations in the presence of sulphuric acid. They are almost certainly responsible for the positive Liebermann-Burchard tests given by members of this family and the positive tests are probably not due to the presence of triterpenes or sterols.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
Coprosma serrulata Hook. f.		L	Herbarium	—	43900		—	—	—	+
Coprosma tenuifolia Cheesem.	soft-leaved coprosma	L	National Park	Oct.	50070	—	—	—	—	++
		W	,,	,,		—		—	—	+
		B	,,	,,		—	—	—	—	+
		R	Ohakune	Nov.		—	—			
Coprosma ocutifolia Hook. f.		L	Auckland	Sept.		—	—	—	—	+
		W, B	,,	,,		—	—	—	—	++
Coprosma australis Robinson	kanono	L	Auckland	Jan.		—	—	—	—	+
		W	,,	,,		—	—	—	—	+
		L	Waitakeres	March		—	—	—	—	++
		W	,,	,,		—	—	—	—	++
		B	,,	,,		—	—	—	—	++
Coprosma robusta Raoul	karamu	L	Auckland	Nov.		—	—	—	(+)	++
		W	,,	,,		—	—	—	—	++
		RW	,,	,,		—	—	—	(+)	++
		B	,,	,,		—	—	—	—	++
		RB	,,	,,		—	—	—	—	++
		F	,,	Jan.		—	—	—	+	++
Coprosma macrocarpa Cheesem.		L	Herbarium	—	37286	—	—	—	—	++
Coprosma petiolata Hook. f.		L	Kermadec Is.	June		—	—	—	—	++
		W, B	,,	,,		—	—	—	—	++
Coprosma repens A. Rich.	taupata (alpine creeping) coprosma	L	Auckland	Sept.		—	—	—	—	++
		W	,,	,,		—	—	—	—	—
Coprosma lucida J. R. et G. Forst.	karamu	Fl	Piha	May		—	—	—	—	+
Coprosma dodonaeifolia W. R. B. Oliver		L	Herbarium	—	40145		—	—	—	++
Coprosma "cunninghamii" (*C. robusta* X *C. propinqua*)		L	Auckland	Sept.		—	—	—	—	—
		W	,,	,,		—	—	—	—	+

Coprosma "neglecta" (*C. repens* X *C. rhamnoides*)		L, T	Herbarium	—	50824		—	—	—	+
Nertera depressa Banks et Sol ex Gaertn.	fruiting duckweed	H	Herbarium	—	36914	—	—	—	—	+
Nertera cunninghamii Hook. f.		H	Waitakeres	March	46455	—	—	—	—	+
Netera dichandraefolia Hook. f.	dichondra-leaved nertera	H	Auckland	Sept.		—	—	—(59)	—	+
Nertera setulosa Hook. f.		H	Herbarium	—	44736	—	—	—(59)	—	+
Galium tenuicaule A. Cunn.	bedstraw	H	Herbarium	—	31849	—	—	—	—	+
Galium propinquum A. Cunn.	mawe	H	Waitakeres	Dec.		—	—		—	—
Galium perpusillum Allan	woodroof	H	Herbarium	—	50490	—	—	—	—	+
COMPOSITAE										
Siegesbeckia orientalis L.	punawaru	H	Herbarium	—	48862	—	—(60)		—(61)	—(61)
Brachycome radicata Hook. f. var. *thomsonii* Allan		H	Herbarium	—	50704	—	—		—	—
Brachycome sinclairii Hook. f.	grass-land daisy	H	Herbarium	—	9369	—	—		—	—
Logenophora pinnatifida Hook. f.		H	Herbarium	—	50827	—	—	—	—	+
Lagenophora petiolata Hook. f.	parani	H	Herbarium	—	32429	—	—		—	—
Lagenophora pumila Cheesem.	papatani-whaniwha	H	Waikaremoana	Nov.		—	—		—	—
Lagenophora pumila Cheesem. var. *barkeri* Simpson		H	Herbarium	—	58907	—	—			—
Celmisia walkeri Kirk		L, St	Herbarium	—	43907	—	—	—	—	+
Celmisia rupestris Cheesem		L, St	Herbarium	—	15601	—	—	—	—	+
Celmisia lateralis Buchan.		L, St	Herbarium	—	44275		—	—	—	—
Celmisia ramulosa Hook. f.		L, St	Herbarium	—	44153	—	—	—	—	+
Celmisia brevifolia Ckn.		L, St	Herbarium	—	34925	+	+	—	—	+
Celmisia sessiliflora Hook. f.	Short-flowered celmisia	L, St	Auckland	Sept.		—	—	—	—	—

(59)Briggs and Nicholls (1954) record negative asperuloside tests. Positive tests have been obtained in the present examination.
(60)Webb (1949) records positive alkaloids tests.
(61)Simes et al. (1959) record negative saponin and triterpene tests.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
Celmisia laricifolia Hook. f.	needle-leaved celmisia	H	Herbarium	—	43905		—	—	—	—
Celmisia discolor Hook. f.	mountain-musk	L, St	Herbarium	—	32650	—	—	—	—	+
Celmisia incana Hook. f.	white mountain musk	L	National Park	Nov.	50122	—	—	—	—	—
		St	„	„	„	—	—	—	—	—
Celmisia haastii Hook. f.		L, St	Herbarium	—	15610	+	+	—	—	—
Celmisia hectori Hook. f.		L, St	Herbarium	—	50953		—	—	—	—
Celmisia viscosa Hook. f.	snow-celmisia	L, St	Herbarium	—	34983	—	+	—	—	+
Celmisia densiflora Hook. f.		L, St	Herbarium	—	34920	—	—	—	—	+
Celmisia glandulosa Hook. f.		H	Waiouru	Oct.	50066	—	—	—	—	—
Celmisia bellidioides Hook. f.	green cushion-celmisia	L, St	Herbarium	—	32451		—	—	(+)	+
Celmisia spectabilis Hook. f.	puakaito	L, St, Fl	Ohakune	Jan.		—	—	—	—	—
		R	„	„		—	—	—	+	—
Celmisia coriacea Hook. f.	tikumu	L, St	Auckland	Sept.		—	—	—	—	—
Celmisia hierocifolia Hook. f.		L, St	Herbarium	—	51045	—	+	—	—	+
Celmisia dallii Buchan.		L, St	Herbarium	—	51043	—	—	—	—	+
Celmisia holosericea Hook. f.	pekepeke	L, St	Herbarium	—	44291	—	—	—	—	+
Celmisio traversii Hook. f.	brown mountain daisy	L, St	Herbarium	—	44024	—	—	—	—	—
Celmisia armstrongii Petrie		L, St	Herbarium	—	32485	—	+	—	—	—
Celmisia lyallii Hook. f.	blunt leaved spaniard	L, St	Herbarium	—	15621	—	+	—	—	—
Celmisia adamsii Kirk		L, St	Herbarium	—	15622	—	+	—	—	—
Celmisia major Cheesem. var. *major*		L, St	Waitakeres	Jan.		—	—	—	—	—
		Fl	„	„		—	—	—	—	—
Celmisia gracilenta Hook. f.	pekepeke	L, St	National Park	Nov.	50143	—	—	—	—	—
Celmisia olpina Cheesem.		L, St	Herbarium	—	32133		—	—	—	—

Celmisia graminifolia Hook. f.		L, St	Herbarium	—	44276		—	—	—	—
Vittadinia australis A. Rich.	southern vittadinia	L, St, Fl	Herbarium	—	70724	—	—([62])	—	—	—
Olearia augustifolia Hook. f.	teteaweka	L	Herbarium	—	35085	—	—			+
Olearia oporina Hook. f.	tupari	L	Herbarium	—	44269	—	—	—	—	+
Olearia chathamica Kirk	keketerehe	L	Herbarium	—	35075	—	—		—	+
Olearia semidentata Decne.	makora	L	Herbarium	—	35074	—	—	—	—	+
Olearia colensoi Hook. f.	tupare	L	Herbarium	—	32566	—	—	—	—	+
Olearia paniculata Druce	akiraho	W	Huia	March		—	—	—	—	+
		B	,,	,,		—	—	—	—	+
Olearia nummularifolia Hook. f.	hard-leaved tree daisy	L	National Park	Nov.	50110	—	—		(+)	+
		W	,,	,,		—	—		—	+
		B	,,	,,		—	—	—	—	+
Olearia nummularifolia Hook. f. var. *cymbifolia* Hook. f.	boat-leaved tree daisy	L	Auckland	Sept.		—		—		+
Olearia moschata Hook. f.	musky daisy tree	L	Herbarium	—	44105	—		—	—	+
Olearia avicenniaefolia Hook. f.	mountain-akeake	L	Auckland	Aug.		—	—	—	—	—
		W	,,	,,		—	—	—	—	+
Olearia albida Hook. f.	Auckland tree daisy	L	Auckland	Aug.		—	—	—([63])	+	—
		W	,,	,,		—	—	—	+	—
Olearia albida Hook. f. var. *angulata* Allan		L	Auckland	Sept.		—		—		—
Olearia allomii Kirk		L	Gt. Barrier Is.	Nov.		—	—		—	—
		W	,,	,,			—		—	—
		F	,,	,,		—	—	—	—	—
Olearia arborescens Ckn. et Laing	daisy-tree	L	Maungatepopo	Nov.	50132	—	—	—	—	
		W	,,	,,		—		—	—	+
		R	,,	,,		—	—	—	—	+
Olearia capillaris Buchan.		L	Herbarium	—	32440		—		—	—
		St	,,	—	,,		—	—	—	—
Olearia furfuracea Hook. f.	akepiro	L	Waitakeres	Feb.		—	—	—	—	—
		W	,,	,,		—	—	—	—	+
		R	,,	,,		—	—	—	—	+
		Fl	,,	,,			—	—	—	

([62])Webb (1952) records positive alkaloid tests.
([63])Bate-Smith and Metcalfe (1957) record the absence of leucoanthocyanins in the leaves.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
Olearia thomsonii Cheesem.		L	Herbarium	—	50255	—	—	—	—	+
Olearia pachyphylla Cheesem.		L	Herbarium	—	48806	—	—	—	—	++
Olearia ilicifolia Hook. f.	hakeke (Maori holly)	L	Auckland	Sept.		—	—	—	—	+
Olearia lacunosa Hook. f.		L	Herbarium	—	44270	—	—	—	—	+
Olearia fragrantissima Petrie	fragrant tree-daisy	L	Herbarium	—	33884	+	+	—	—	—
Olearia rani Druce	heketara	L	Waitakeres	Sept.		—	—	—	+	+
		Fl	,,	,,		—	—	(+)	+	—
Olearia traversii Hook. f.	Chatham akeake	L	Herbarium	—	36100	—	—	—	—	+
Olearia virgata Hook. f.	swamp tree-daisy	L	Herbarium	—	61957	—	—	—	—	-
Olearia virgata Allan var. *lineata* Kirk	twiggy tree-daisy	L, Fl	Herbarium	—	33896	—	—	—	—	
Olearia solandri Hook. f.	coastal daisy-tree	L	Herbarium	—	50861		—	—	—	+
		W, B	,,	—	,,		—	—	—	++
		Fl	,,	—	,,		—	—	—	+
Pachystegia insignis Cheesem.	rock daisy-tree	L, St	Auckland	Sept.		—	—	—	—	—
Haastia recurva Hook. f.		L, St	Herbarium	—	32198		—	—	—	—
Haastia sinclairii Hook. f.		L, St	Herbarium	—	43904		—	—	—	—
Cotula coronopifolia L.	yellow-button	L, St	Wellington	Sept.		—	—	—	—	—
Cotula atrata Hook. f.	black daisy	H	Herbarium	—	32038	—	—	—	—	-
Cotula minor Hook. f.		H	Herbarium	—	32039	+	+	—	—	—
Cotula pyrethrifolia Hook. f.	mountain-cotula	H	Herbarium	—	22374	—	—	—	—	-
Cotula squalida Hook. f.		H	Herbarium	—	65405	—	—	-	-	-
Cotula perpusilla Hook. f.		H	Herbarium	—	50516	—	—	—	—	(+)

Cotula dioica Hook. f.	shore-cotula	H	Herbarium	—	65406	—	—	—	—	—
Abrotanella linearis Bergg.		L, St	Herbarium	—	32425	—	—	—	—	—
Gnaphalium trinerve Forts. f.	tree-nerved cudweed	L, St	Herbarium	—	32228	—	—	—	—	—
Gnaphalium keriense A. Cunn.	river-daisy	L, St	Waitakeres	Dec.		—	—	—	—	—
		Fl	,,	,,		—	—	—	—	—
		L	Auckland	Sept.		—	—	—	—	—
Gnaphalium subrigidum Col.		L	Auckland	Sept.		—	—	—	—	—
Gnaphalium traversii Hook. f. var. *mackayi* Kirk		L, St	Herbarium	—	44051		—	—	—	—
Gnaphalium paludosum Petrie	marsh cudweed	L, St	Herbarium	—	32459		—	—	—	—
Gnaphalium luteo-album L.	flannel-leaf	L, St	Herbarium	—	70640		(64)	—	—	—
		Fl	,,	—	,,		—	—	—	(+)
Gnaphalium collinum Labill	hill-cudweed	L, St	Herbarium	—	70112		—	—	—	—
Raoulia australis Hook. f.	common raoulia	H	Taupo	Oct.	46530 & 46531	—	—	—	—	—
		H	Herbarium	—	50858		—	—	—	—
Raoulia subsericea Hook. f.	turfy raoulia	H	Herbarium	—	46137		—	—	—	—
Raoulia glabra Hook. f.	glabrous raoulia	H	Herbarium	—	66728		—	—	—	—
Raoulia tenuicaulis Hook. f.	tutahuna	H	Herbarium	—	58386		—	—	—	—
Raoulia monroi Hook. f.		H	Herbarium	—	48839		—	—	—	—
Raoulia grandiflora Hook. f.	large-flowered raoulia	H	Herbarium	—	32596		—	—	—	—
Raoulia hectori Hook. f.		H	Herbarium	—	70186		—	—	—	—
Raoulia eximia Hook. f.	vegetable-sheep	H	Herbarium	—	45300		—	—	—	—
Raoulia buchananii Kirk		H	Herbarium	—	32310		—	—	—	—
Leucogenes leontopodium Beauverd	Maori edelweiss	H	Herbarium	—	70642		—	—	—	—
Leucogenes grandiceps Beauverd	edelweiss	H	Herbarium	—	32563		—	—	—	—
Ewartia sinclairii Cheesem.		H	Herbarium	—	32374		—	—	—	—
Helichrysum bellidioides Willd.	mountain daisy	L, St	Auckland	Sept.		—	—	—	—	—
Helichrysum filicaule Hook. f.	slender everlasting	L, St	Herbarium	—	44013		—	—	—	—

(64)Webb (1952) records positive alkaloid tests.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
Helichrysum dimorphum Ckn.		L, St	Herbarium	—	31937		—	—	—	—
Helichrysum glomeratum Benth.	niniao	L, St	Herbarium	—	58267		—	—	—	—
et Hook. f.		Fl	,,	—	,,		—	—	—	—
Helichrysum depressum Benth. et Hook. f.		L, St	Auckland	Sept.		—	—	—	—	—
Helichrysum microphyllum Benth. et Hook. f.		L, St	Herbarium	—	44021		—	—	—	—
Helichrysum selago Benth. et Hook. f.	selago-like everlasting	L	Auckland	Sept.		—	—	—	—	—
Helichrysum coralloides Benth. et Hook f.	coral-shrub	L, St	Herbarium	—	31930		—	—	—	—
Craspedia uniflora Forst. f.	puatea	H	Wellington	Dec.		—	—	—	—	—
Cassinia retorta A. Cunn.		L, St	Auckland	March		—	—	—	—	—
		W	,,	,,		—	—	—	—	—
		F	,,	,,		—	—	—	—	—
Cassinia leptophylla R. Br.	tauhinu-korokio (fragrant cottonwood)	L, St, Fl	Herbarium	—	31970		—	—	—	—
Cassinia amoena Cheesem.		L, St	Auckland	Sept.		—	—	—	—	—
Cassinia vauvilliersii Hook. f.	mountain-cottonwood	L, T	Ohakune	Jan.	44404	—	—	—	—	—
		W, B	,,	,,			—	—	—	—
		RW, RB	,,	,,			—	—	—	—
Cassinia fulvida Hook. f.	golden cottonwood	L, St	Herbarium	—	50512	—	—	—	—	+
Erechtites diversifolia Petrie	Petrie's fireweed	L, St, Fl	Herbarium	—	70639	—	+	—	—	—
Erechtites scaberula Hook. f.	scabrid fireweed	L, St	Herbarium	—	50799	—	—	—	—	—

Erechtites arguta DC.	woolly fireweed	L, St	Herbarium	—	43879	+	+	—	—	—
Erechtites quadridentata DC.	pekapeka	L, St	Herbarium	—	65404	—	+(65)	—	—	—
Erechtites wairauensis Allan	common fireweed	L, St	Herbarium	—	10488 & 15706	+	+	—	—	—
Senecio bellidioides Hook. f.	Maori groundsel	L	Auckland	Sept.		++	++	—	—	—
		L, Fl	Herbarium	—	32464		—	—	—	—
		St	,,	—	,,		—	—	+	—
Senecio haastii Hook. f.		L, St	Herbarium	—	44112	+	+	—	—	—
Senecio lagopus Raoul		L, St	Auckland	Sept.		—	—	—	—	—
		,,	Wellington	Jan.		—	—	—	—	—
Senecio lyallii Hook. f.	white marigold	L, St	Herbarium	—	24314	—	—	—	—	—
Senecio scorzoneroides Hook. f.	snow-groundsel	L, St	Herbarium	—	33767	—	—	—	—	—
Senecio rufiglandulosus var. *solandri* Allan		L	Herbarium	—			—	—	—	—
		Fl	,,	—		—	+	—	—	—
Senecio turneri Cheesem.		L, St	Herbarium	—	35358	—	—	—	—	—
Senecio banksii Hook. f.	East Cape groundsel	L, St	Herbarium	—	43866	—	—	—	—	—
Senecio hectori Buchan.	deciduous tree-groundsel	L, St	Herbarium	—	65408	—	—	—	—	—
Senecio kirkii Hook. f. ex Kirk	tapairu	L	Waitakeres	March		++	++(66)	—	—	—
		W	,,	,,			—			
		B	,,	,,		+	+			
		RW, RB	,,	,,		+	+			
Senecio myrianthos Cheesem.		L	Herbarium	—	15739	—	—	—	—	—
Senecio adamsii Cheesem.		L	Herbarium	—	50395	-	+	—	—	+
Senecio cassinioides Hook. f.		L	Herbarium	—	44117	+	+	—	—	+
Senecio laxifolius Buchan.		L	Herbarium	—	15743	+	+	—	—	+
Senecio greyi Hook. f.		L	Auckland	Aug.		+	+	—	—	+
Senecio perdicioides Hook. f.	raukumara	L	Herbarium	—	15740	—	—	—	—	+
		Fl	,,	—	,,	—	—	—	—	+
Senecio compactus Kirk		L	Auckland	Sept.		—	—	—	—	—
		W	,,	,,		—	—	—	—	+

(65)Webb (1952) records negative alkaloid tests on a 19 year old herbarium sample.
(66)Briggs, Mangan, and Russell (1948) have isolated an alkaloid senkirkine, of unknown structure, from the bark and leaves.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
Senecio reinoldii Endl.	puheretaiko	L, T, Fl	Herbarium	—	35279	–	–	–	–	–
Senecio elaeagnifolius Hook. f.	brown-backs	W	Stewart Is.	Jan.	44394	–	+	–	–	
		B	,,	,,	,,	++	++	(+)	–	+
Senecio bidwillii Hook. f.	Bidwell's shrubby groundsel	L	Ohakune	Jan.	44417	–	–	–	–	–
		W	,,	,,		–	–	–	–	–
		B	,,	,,		–	–	–		–
Brachyglottis repanda J. R. et G. Forst.	rangiora	L	Kawakawa	Jan.		++	++(67)	–	–	–
		W	,,	,,		++	++	–	–	–
		B	,,	,,		+	+	–	–	–
		RW, RB	,,	,,		+	++	–	–	–
		Fl	Waitakeres	Aug.		+	+	–	–	–
Brachyglottis repanda J. R. et G. Forst. var. *rangiora* Allan	rangiora	L	Auckland	Sept.		–	+	–	–	–
		W	,,	,,		–	+	–	–	–
Traversia baccharoides Hook. f.		L, St, Fl	Herbarium	—	44015	–	–	–	–	–
Sonchus littoralis Allan	coastal sow-thistle	L, St	Herbarium	—	50829	+	+	–	–	–
Microseris scapigera Sch. Bip.		L, St	Herbarium	—	44026	–	–	–	–	–
GENTIANACEAE										
Liparophyllum gunnii Hook. f.		H	Herbarium	—	70643	-	–	–	(+)	–
Gentiana grisebachii Hook. f.	common N.Z. gentian	L, Fl	Herbarium	—	61992	+	+	–	–	–
Gentiana saxosa Forst. f.	shore-gentian	L, St	Herbarium	—	46568		–	–	–	–
Gentiana bellidifolia Hook. f.	mountain gentian	L	Herbarium	—	70638	–	–	–	–	++
		Fl	,,	—	,,	–	–	–	–	++
Gentiana patula Cheesem.		L, Fl	Herbarium	—	50853		–	–	–	–
Gentiana corymbifera Kirk	snow-gentian	L, Fl	Herbarium	—	51047		–	–		

PRIMULACEAE										
Samolus repens Pers.	maakoako	H	Auckland	April		—	—([67])	+	(+)	—
PLANTAGINACEAE										
Plantago triantha Spreng.	Brown's plantain	L, St	Herbarium	—	44049	—	—	—	—	
Plantago triandra Bergg.	glossy plantain	L, St	Herbarium	—	32564	—	—	—	—	—
Plantago spathulata Hook. f.	kawpare-rarera	L, St	Herbarium	—	15480	—	—	—	—	—
Plantago raoulii Decne	kopakopa	St, S	Piha	May		—	—	—	—	—
CAMPANULACEAE										
Wahlenbergia albomarginata Hook.	Maori blue-bell	L, St	Herbarium	—	70644		—		—	—
		Fl	,,	—	,,		—	—	—	
DONATIACEAE										
Donatia novae-zelandiae Hook. f.	alpine donatia	H	Herbarium	—	24296	—	—	—	—	—
GOODENIACEAE										
Selliera radicans Cav.	remuremu	H	Waitakeres	Jan.			—	(+)	—	—
Scaevola gracilis Hook. f.		T	Kermadec Is.	June		+	++	—	—	—
LOBELIACEAE										
Pratia physaloides Hemsl.		L	Herbarium	—	70638		—	—	—	
		W	,,	—	,,		—	—	—	
Pratia angulata Hook. f.	panakenake	H	Herbarium	—	46464		—	—	—	—
Pratia perpusilla Hook. f.		H	Herbarium	—	50982		—	—	—	—
Lobelia anceps Linn. f.	shore-lobelia	H	Herbarium	—	44885			—	—	—

([67])Briggs (1947) reports the presence of alkaloids in the leaves.

([68])Anomalous tests. Dragendorff's reagent gave a black precipitate and Mayer's reagent gave a pink colour changing to blue but no precipitate.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
STYLIDIACEAE										
Phyllachne colensoi Bergg.	common phyllachne	H	Herbarium	—	35009		—	—	-	—
Phyllachne rubra Cheesem.		H	Herbarium	—	44293		—	+	—	—
Forstera sedifolia Linn. f.	common forstera	L	Herbarium	—	44181	—	—	+	—	+
Forstera bidwillii Hook. f.		L, Fl	National Park	Jan.		—	—	+	—	(+)
		,,	Herbarium	—	32461	—	—	+	—	+
Forstera tenella Hook. f.		L, St	Herbarium	—	51042		—	—	—	—
Oreostylidium subulatum Bergg.		H	Herbarium	—	32438	+	+	—	-	—
BORAGINACEAE										
Myosotidium hortensia Baill.	kopukapapuku (Chatham forget-me-not)	L, Fl	Auckland	Sept.		—	—		—	
Myosotis pulvinaris Hook. f.		L, St	Herbarium	—	46584		—	—	—	—
Myosotis colensoi Macbride		L, St	Herbarium	—	50484		—	—	—	
Myosotis pygmaea Col.		L, St	Herbarium	—	47777		—	—	—	—
Myosotis australis R. Br.	yellow forget-me-not	L, St	Herbarium	—	15357		—	—	(+)	-
Myosotis forsteri Lehm.	forget-me-not	H	Herbarium	-	70641		-	—	—	—
Myosotis amabilis Cheesem.		L, St	Herbarium	—	47774		-	—	—	—
Myosotis rakiura L. B. Moore	coast forget-me-not	L, St	Herbarium	—	46596		-	-	—	-
Myosotis spathulata Forst, f.	forget-me-not	L, St	Herbarium	-	46607		—		.	—

SOLANACEAE										
Solanum aviculare Forst. f.	poroporo	L	Auckland	Jan.		++	+(69)	–	+	+
		F	,,	,,		+++	++	–	+	+
Solanum laciniatum Ait.	poroporo tanguru	F	Dunedin	Jan.		+++	+(70)		+	+
Solanum nodiflorum Jacquin		F	Dunedin	Jan.		+	+(70)	–	+	+
CONVOLVULACEAE										
Calystegia soldanella R. Br.	sandbine	Rh	Auckland	Sept.		–				
Calystegia tuguriorum R. Br. ex Hook	powhiwhi (Maori convolvulus)	Rh	Herbarium	—	50587		–	–	–	–
Ipomoea palmata Forsk.	panahi	H	Herbarium	—	24405	+	+	–	–	–
SCROPHULARIACEAE										
Jovellana sinclairii Kranzl.	Maori calceolaria	L	Auckland	Aug.				–		
		W	,,	,,			–	–	–	–
Jovellana repens Kranzl.	creeping N.Z. calceolaria	L, St	Herbarium	—	44748		–	–		
Gratiola sexdentata R. Cunn.		H	Herbarium	—	58355		–	–	(+)	
Glossostigma elatinoides Benth.	elatine-like glossostigma	H	Herbarium	—	50842		–	–	(+)	
Limosella lineata Gluck		H	Herbarium	—	44830	–	+	–	–	
Mimulus repens R. Br.	Maori musk	H	Herbarium	—	2406	–	+	–	(+)	–
Mozus radicans Cheesem.		L, St	Herbarium	—	32571		–	–	(+)	
Euphrasia cuneata Forst. f.	tutumako	H	National Park	Jan.					–	–
Euphrasia monroi Hook. f.	alpine eye-bright	H	Herbarium	—	65423		–			–
Euphrasia laingii Petrie		H	Herbarium	—	598		–	–	–	–
Euphrasia revoluta Hook. f.		H	Herbarium	—	44289		–	–	–	–
Euphrasia townsonii Petrie		H	Herbarium	—	28141		–	–	–	

(69)Briggs and Bell (1942), Briggs and Cambie (1958b), Briggs, Cambie, and Hoare (1961, in press), Kuhn, Low and Trischmann (1955) have shown the presence of the alkaloids, solasonine and solamargine in the fruit and leaves. Webb (1949, 1952) records positive alkaloid tests for the pith, leaves and bark but negative alkaloid tests for the wood and mature fruit.

(70)Briggs and Cambie (1958b) have recorded the isolation of the alkaloid solasonine from the fruit.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
Euphrasia zelandica Wettst.	Maori eye-bright	H	Herbarium	—	70386		—	—	—	—
Euphrasia disperma Hook. f.	slender flowered eyebright	H	Herbarium	—	8593		—	—	—	—
Ourisia macrophylla Hook.	hue-o-Raukatauri (mountain-foxglove)	L	Herbarium	—	61900		—	—([71])	—	—
Ourisia colensoi Hook. f.	Colenso's ourisia	H	National Park	Nov.	50118	—	—	—	—	(+)
Ourisia macrocarpa Hook. f.	snowy mountain-foxglove	L	Herbarium	—	32520		—	—	—	—
Ourisia caespitosa Hook. f.	creeping mountain-foxglove	H	Herbarium	—	70385		—	—	—	—
Pygmea pulvinaris Hook. f.		H	Herbarium	—	44296		—	—	—	—
Parahebe lyallii W. R. B. Oliver		H	Herbarium	—	70302		—	—	—	—
Parahebe catarractae W. R. B. Oliver	waterfall koromiko	L	National Park	Jan.		—	—	—	—	—
Parahebe hookeriana W. R. B. Oliver	Hooker's veronica	L	National Park	Jan.		—	—	—	—	—
		St	,,	,,			—	—	—	—
		S	,,	,,			—	—	—	—
Parahebe linifolia W. R. B. Oliver	wet-rock koromiko	L	Herbarium	—	44048		-	—	—	
Parahebe spathulata W. R. B. Oliver	scoria-veronica	L	Herbarium	—	58392		—	—	—	—
Parahebe canescens W. R. B. Oliver		H	Herbarium	—	15448		—	—	—	—

Hebe diosmifolia Ckn. et Allan	heath-koromiko	L	Auckland	Sept.		+	+	–	+	–
		W	,,	,,		+	+	–	+	–
		S	,,	,,		–	+	–	–	–
Hebe diosmifolia Ckn. et Allan var. *trisepala* Kirk		L	Auckland	Sept.		–	–	–	+	–
		W	,,	,,		–	+	–	+	–
Hebe rigidula Ckn. et Allan		L	Herbarium	—	32612		–	–	–	–
Hebe venustula L.B. Moore		L	Herbarium	—	50839	–	+	–	–	–
Hebe canterburiensis L. B. Moore		L	Herbarium	—	70632		–	–	–	–
Hebe elliptica Pennell	shore-koromiko	L	Auckland	Jan.	44409		–	–	–	–
Hebe speciosa Ckn. et Allan	napuka	L	Auckland	Aug.		–	–	–	–	–
		St	,,	,,		–	–	–	–	–
		Fl	,,	Aug.		–	–	–	–	–
Hebe townsonii Ckn. et Allan		L	Auckland	Aug.			–	–	+	–
		W	,,	,,			–	–	+	–
Hebe salicifolia Pennell	willow-koromiko	L	Huia	March	46485 &		–	–	–	–
		W, B	Waitakeres	Aug.	46453	(+)	(+)	–	–	–
		RW	Huia	March			–	–	–	–
		RB	,,	,,		–	–	–	–	–
		Fl	Waitakeres	Aug.		(+)	(+)	–	–	–
		S	Huia	March			–	–	–	–
Hebe pubescens Ckn. et Allan		L, T	Herbarium	—	15404		–	–	–	–
Hebe ligustrifolia Ckn. et Allan		L, T	Herbarium	—	50817	+	+	–	+	–
Hebe stricta L. B. Moore var. *macroura* L. B. Moore		L	Herbarium	—	50179	–	–	–	+	–
		L	Auckland	Sept.		–	–	–	+	–
		St	,,	,,		–	–		–	–
Hebe obtusata Ckn. et Allan		L	Herbarium	—	50175		–	–	+	–
Hebe macrocarpa Ckn. et Allan		L	Waitakeres	March	46452	–	–	–	–	–
Hebe macrocarpa Ckn. et Allan var. *latisepala* Ckn. et Allan		L	Auckland	Aug.		–	–	–	+	–
		W	,,	,,		–	–	–	+	–
Hebe bollonsii Ckn. et Allan		L	Auckland	Aug.		–	–	–	+	–
		St	,,	,,		–	–	–	+	–
Hebe barkeri Ckn.		L	Auckland	Aug.		–	–	–	–	–
		St	,,	,,		–	–	–	–	–
Hebe evenosa Ckn. et Allan		L	Auckland	Aug.		–	–	–	–	–
		St	,,	,,		–	–	–	–	–

([71])Bate-Smith and Metcalfe (1957) record the absence of leucoanthocyanins in the leaves.

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
Hebe parviflora Ckn. et Allan	koromiko-taranga	L	Auckland	Aug.		—	(+)	—	+	—
		W	,,	,,		—	—	—	++	—
Hebe parviflora Ckn. et Allan var. *angustifolia* L. B. Moore		L	Auckland	Aug.		—	—	—	++	—
		W	,,	,,		—	-	—	+	—
Hebe subalpina Ckn. et Allan	subalpine koromiko	L	Auckland	Sept.		—	—	—	-	—
Hebe glaucophylla Ckn.		L	Auckland	Aug.		—	—	—	+	—
		W	,,	,,		—	—	—	++	—
Hebe decumbens Ckn. et Allan		L	Auckland	Aug.		—	—	—	++	—
		W	,,	,,		—	—	—	+	—
		S	,,	,,		—	—	—	-	—
Hebe amplexicaulis Ckn. et Allan		L, T	Herbarium	—	70370		—	—	+	—
Hebe pinguifolia Ckn. et Allan		L	Auckland	Sept.		—	—	—	-	—
		L	Herbarium	—	32645		-	—	-	—
Hebe buchananii Ckn. et Allan		L, T	Herbarium	—	50969		—	—	+	—
Hebe odora Ckn.		L	Waiouru	Oct.	50069	—	—	-	-	—
		W	,,	,,		(+)	(+)	—	—	—
		B	,,	,,		—	—	—	-	—
Hebe "anomala" Ckn.		L	Auckland	Aug.		—	—	—	+	—
		W	,,	,,		—	—	—	++	—
Hebe tetragona Ckn. et Allan	whipcord koromiko	L	Waiouru	Oct.	50063 &	—	—	—	-	—
		W	,,	,,	50112	+	—	—	-	—
		B	,,	,,		—	—	-	-	—
Hebe hectori Ckn. et Allan		L	Auckland	Aug.		—	-	—	-	—
		W, B	,,	,,		—	—	—	-	-
		L	Herbarium	—	70384		—	—	-	-
Hebe lycopodioides Ckn. et Allan	whipcord koromiko	L	Herabrium	—	70383		—	—	-	-
Hebe salicornioides Ckn. et Allan		L	Herbarium	—	8241		—	—	+	—

Hebe cupressoides Ckn. et Allan	cypress-koromiko	L	Auckland	Aug.		–	–	–	–	–
		S	,,	,,		–	–	–	–	–
		L	Herbarium	—	15427		–	–	–	–
Hebe epacridea Ckn. et Allan	trailing-koromiko	L	Herbarium	—	15430		–	–	–	–
Hebe lavaudiana Ckn. et Allan	Lavaud's koromiko	L	Auckland	Aug.		–	+	–	–	–
		W	,,	,,		–	+	–	–	–
Hebe hulkeana Ckn. et Allan	lilac-koromiko	L	Auckland	Sept.		–	–	–	–	–
Hebe macrantha Ckn. et Allan		L	Herbarium	—	43901		–	–	–	–
Hebe tetrasticha Ckn. et Allan	semi-whipcord koromiko	H	Herbarium	–	70381		–	–	–	–
Hebe "lewisii" J. B. Armst.		L	Auckland	Aug.		–	–	–	+	–
		W	,,	,,		–	–	–	+	–
Hebe "andersonii" Ckn.		L	Auckland	Aug.		–	+	–	+	–
		W	,,	,,		–	(+)	–	+	–
Hebe "balfouriana" Ckn.		L	Auckland	Aug.			+	–	+	–
		W	,,	,,			+	–	+	–
Hebe "carnea" Hort. ex J. B. Armst.		L	Auckland	Aug.		+	+	–	+	–
		W	,,	,,		(+)	+	–	+	–
		Fl	,,	,,		(+)	+	–	–	–
BIGNONIACEAE										
Tecomanthe speciosa W. R. B. Oliver		L	Herbarium	—	42594		–	–	–	–
		L	Auckland	Aug.		–	+	–	–	–
		W	,,	,,		–	+	–	–	–
		B	,,	,,		–	+	–	–	–
GESNERIACEAE										
Rhabdothamnus solandri A. Cunn.	taurepo	L	Karekare	March	46467		–		–	–
		R	,,	,,			–	–	–	–
LENTIBULARIACEAE										
Urtricularia protrusa Hook. f.	floating bladderwort	H	Herbarium	—	35766		–	–	–	–
Urtricularia delicatula Cheesem.		H	Herbarium	—	70387		–	–	–	–

TABLE 1—*continued*

Systematic Name of Plant	Maori Name or Popular Name	Plant Part	Locality	Month Collected	Herbarium No.	Alkaloid Test M	Alkaloid Test D	LA	Sap	LB
Urtricularia novae-zelandiae Hook. f.		H	Herbarium	—	35806		—	—	—	—
Urtricularia monanthos Hook. f.	purple bladderwort	H	Herbarium	—	32484		—	—	—	—
MYOPORACEAE										
Myoporum laetum Forst. f.	ngaio	T	Huia	March		++	+++	−(72)	—	—
		W	,,	,,		—	—	—	—	—
		RW	,,	,,		—	—	—	—	—
		B	,,	,,		+++	+++	—	—	—
		RB	,,	,,		—	—	—	—	—
VERBENACEAE										
Vitex lucens Kirk	puriri	L	Kawakawa	Dec.		—	+(73)	—	—	—
		W	,,	,,		—	+	—	—	—
		RW	,,	,,		—	+	—	—	—
		RB	,,	,,		—	—	—	—	—
		Fl	Waitakeres	June		—	—	—	—	+
		F	Kawakawa	Dec.		—	—	—	—	—
Teucridium parvifolium Hook. f.		L, T	Herbarium	—	70388		—	—	—	—
AVICENNIACEAE										
Avicennia resinifera Forst. f.	manawa (mangrove)	L, St	Auckland	Jan.		++	++	—	—	—
		W	,,	,,		+	+	+	—	—
		RW	,,	,,		—	—	+	—	—
		B	,,	,,		+	+	—	—	—
		RB	,,	,,		—	+	—	—	—
LABIATAE										
Mentha cunninghamii Benth.	hioi (Maori mint)	H	Herbarium	—	58372		—	—	-	

(72)Bate-Smith and Metcalfe (1957) record the absence of leucoanthocyanins in the leaves.
(73)Cambie (1959b) records the presence of traces of alkaloids in the leaves and their absence in the bark.

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NOTOTHENIOID FISHES FROM CAPE HALLETT AND ROSS SEA, ANTARCTICA

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(Received for publication, 14 March 1961)

Summary

A systematic list is given of seven fishes collected from shore or from sea ice in the vicinity of Cape Hallett in the period 5 December 1958 to 16 January 1959.

INTRODUCTION

During the southern summer of 1958–59 a programme of collecting of fishes was conducted in the Cape Hallett area of Victoria Land as part of IGY Project 0·4, of the U.S. National Committee, International Geophysical Year, National Academy of Sciences.

Collecting was carried out by the author with occasional help by base personnel. At five stations catches were made by trap, net, and line where the fish could be seen at the bottom and lured into the net. The water at the sixth station (Station A), one of the most productive, was completely clouded with drifting silt, organisms, and stained runoff from the penguin rookery, and fishing with net and trap was conducted "blind". All sites were in the immediate vicinity either of Cape Hallett or Hallett Inlet and the Joint New Zealand - United States Scientific Station, which lies in latitude 72° 18′ S, on the western shore of the Ross Sea (Figs. 1, 2).

RESULTS

A total of three hundred and nineteen nototheniid fishes was obtained. These have been referred to six species of the genus *Trematomus* and one of the genus *Notothenia*. A systematic list is given in Table 1.

The collecting localities ranged from a sheltered ice-covered cove (Station A) to open current-swept and berg-scarred channel, as follows: Site A, an artificial hole blasted in full cover of bay ice of Willett Cove (depth 3–4 metres); Site B, a natural melt-hole at the side of entrapped bergy bit, over a gravel-shingle bottom (depth 6–8 metres); Site C, the icefoot of beach at station landing after the spring break-up (depth to one metre); Site C_1, a similar icefoot at Hallett Headland; and Site D, a close offshore area, open during later days of the sampling but frequently ice-beset during flood tide (depths 6–15 metres). The hole at the bergy bit (B) produced all seven species, the single specimen of *Trematomus borchgrevinki* being the only non-benthic form. All of the fish observed or captured in these shallows are sub-adult in size. The series obtained within these several species provides data on habitat and food habits, and distribution of food organisms, to be reported elsewhere (Miller, in press).

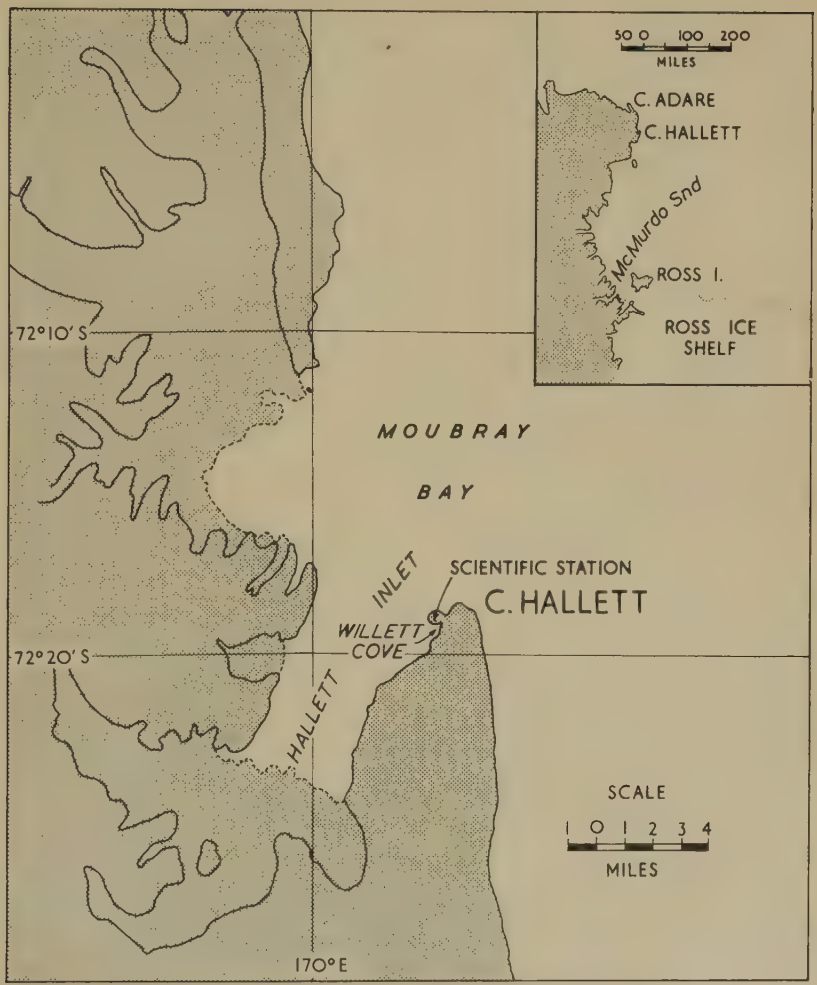

FIG. 1—Locality chart.

DISTRIBUTION

During the 1959–60 study of the fishes of the Ross Sea reported here and by Reseck (1961) twenty-eight species of fishes have been identified. These represent 80·0% of the species known in the Ross Sea fauna. Nineteen species are represented by two or more examples.*

*In addition to these collections from the Ross Sea, our collection now includes some ninety specimens from McMurdo Sound and a lot, of similar size, from Wilkes Station in Vincennes Bay, as well as collections representing some fourteen other species from seven other areas of the Antarctic. Some sixty-five specimens were obtained in the 1959–60 season when the author was exchange observer for the U.S. Antarctic Projects Office, with the Argentine Campaign.

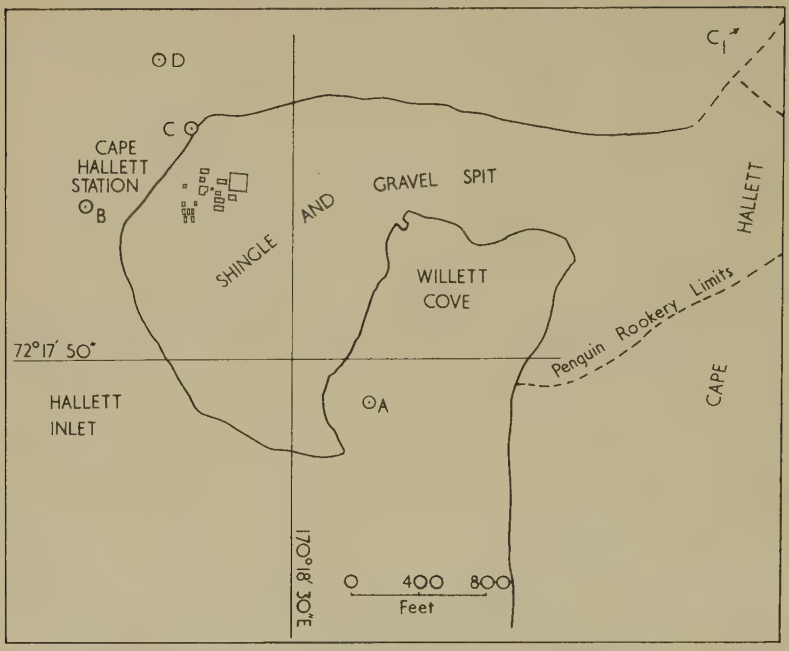

FIG. 2—Collecting sites near Cape Hallett.

The preponderance of the collections reported here falls into four families, Nototheniidae, Harpagiferidae, Bathydraconidae, and Chaenichthyidae, which together constitute the greater part of the Nototheniiform group of the Percoidea. Such fishes are large of head and fins, with the pelvic fins jugular, with no pungent spines in fins, and with three radials present in the pectorals.

The Nototheniiform fishes possess the characteristics of bottom-dwellers and apparently most of them are truly benthic in habit. All of the species occurred exclusively in bottom situations except the following three:

1. *Trematomus borchgrevinki* was taken with a dip-net at the bay ice underface;
2. *Notothenia coriiceps* was taken near the beach where it was enticed from the cavernous icefcot;
3. One specimen of *Pagetopsis macropterus* occurred in a vertical plankton haul which originated considerably below the previously known depths for this fish, a fact suggesting this specimen may have been swept up in mid-water.

Of the other two families, the Zoarcidae has numerous genera in both northern and southern oceans which are generally deep-sea in occurrence.

Two species are found in Antarctic coastal waters, both now known from the Ross Sea, two examples of *Austrolycichthys concolor* having occurred in the *Endeavour* catches.

The remaining family, *Muraenolepidae,* four species of which occur in the sub-Antarctic and Antarctic seas, is a distinctive segment of the large and widespread order, Gadiformes. In the Ross Sea fauna there is one species, *Muraenolepis microps,* of which Mr Reseck secured a specimen.

TABLE 1—List of Fishes Collected near Cape Hallett

H1 to H327 are field collection numbers.
The range of standard lengths for each station is given for each species.
The collecting localities are identified by the letters A, B, C, C_1, and D (see text). They are shown here with the yield from each and the size ranges of the fish.

Family NOTOTHENIIDAE	
1. *Notothenia coriiceps* Richardson	*26 spms, 67–81 mm.*
	A = 0.
H79, 128, 129	B: 3 spms, 67–73 mm.
H229, 230, 234–240, 284–288, 288A	C: 15 spms, 67–81 mm.
H239, 308, 310	D: 3 spms, 70–73 mm.
H320–324	C_1: 5 spms, 71–76 mm.
2. *Trematomus borchgrevinki* Boulenger	*1 spm.*
H1	B: 42 mm.
3. *Trematomus newnesi* Boulenger	*9 spms, 45–72 mm.*
H81A, 94	B: 2 spms, 65–72 mm.
H259, 298, 325, 326	C: 5 spms, 45–71 mm.
H218, 233	D: 2 spms, 50–60 mm.
4. *Trematomus nicolai* (Boulenger)	*3 spms, 67–86 mm.*
H11, 69	B: 2 spms, 70–86 mm.
H246	C: 1 spm, 67 mm.
5. *Trematomus bernacchii* Boulenger	*43 spms, 67–86 mm.*
H2, 4–10, 13, 15–23, 21A, 26, 28, 29, 32, 61–67, 72, 80, 96	B: 32 spms, 54–118 mm.
H123, 124A, 130, 139, 149–151, 186A, 305, 327	A: 10 spms, 49–153 mm.
H227	D: 1 spms. 50 mm.
6. *Trematomus centronotus* Regan	*51 spms, 43–112 mm.*
H3, 11 (/12), 66, 68, 70, 73, 74, 74A, 75–78 81	B: 13 spms, 43–85 mm.
H85, 131, 132, 134–138, 143–148, 152, 153, 155, 157–161, 170–171, 174, 178, 189, 219–224, 226–228, 301A, 304, 306, 307	A: 38 spms, 49–112 mm.
7. *Trematomus pennellii* Regan	*186 spms, 37–108 mm.*
H14, 27, 30, 31, 33–36, 38–56, 58–60, 71, 83, 84, 86, 87, 90–93, 95, 97–107, 109–122, 277–283	B: 73 spms, 48–108 mm.
H124–126, 133, 141–142, 154, 156, 162–169, 172, 173, 175–177, 179–185, 188, 190, 191, 300	A: 32 spms, 51–102 mm.
H241, 242, 244, 245, 247A–258, 260–276, 289, 297, 302. 303	C: 44 spms, 37–62 mm.
H187, 192–212, 214–217, 231, 232, 311–319	D: 37 spms, 39–80 mm.

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